AD_____

AN EXPERIMENTAL BRAIN MISSILE WOUND: ASCERTAINING PATHOPHYSIOLOGY AND EVALUATING TREATMENTS TO LOWER MORTALITY AND MORBIDITY

ANNUAL REPORT

MICHAEL E. CAREY
DAN TORBATI
JOSEPH SOBLOSKY
JUNE DAVIDSON
J. BRYAN FARRELL

OCTOBER 26, 1990

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21702-5012

Contract No. DAMD17-86-C-6098

Louisiana State University Medical Center
1542 Tulane Avenue
New Orleans, Louisiana 70112

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

1a. REPORT SECURITY CLASSIFICATION: Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT: Apprcved for public release; distribution unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION: Louisiana State University Medical Center; 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code): 1542 Tulane Avenue, New Orleans, LA 70112-2822	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION: US Army Medical Research & Development Command; 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: DAMD17-86-C-6098
8c. ADDRESS (City, State, and ZIP Code): Fort Detrick, Frederick, MD 21702-5012	10. SOURCE OF FUNDING NUMBERS: PROGRAM ELEMENT NO. 62787A; PROJECT NO. 3M1-62787A874; TASK NO. CC; WORK UNIT ACCESSION NO. 131

11. TITLE (Include Security Classification)
An Experimental Brain Missile Wound; Ascertaining Pathophysiology and Evaluating Treatments to Lower Mortality and Morbidity

12. PERSONAL AUTHOR(S) Carey, Michael E; Torbati Dan; Soblosky Joseph; Davidson June; Farrell J Bryan

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Annual	FROM 4/14/88 TO 4/13/89	90/10/26	

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	Experimental Brain Wounding; Chemical Brain Blood Flow Regulation After Wounding; Physiological Variables Accounting for Death After Brain Wounding; Lung Water
06	04		
06	14		

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

1) Chemical regulation of CBF: Normoxic hypocapnia ($PaCO_2$ 21 mmHg) significantly reduced total CBF and many rCBFs both before and after wounding but after wounding this response may have been attenuated except around the wound track where CBF reduction was enhanced. Hyperoxia (PaO_2 > 280 mmHg) hetereogeneously altered rCBF in normal brains: cerebral cortex (-7%) brain stem and cerebellum (+40%). After wounding hyperoxia enhanced some rCBF decreases (-15%) but the flow increases in the brain stem and cerebellum were totally abolished. Hyperoxia plus hypercapnia (PaO_2 > 440 mmHg; $PaCO_2$ ~53 mmHg) dramatically increased CBF in normal brains but this effect was greatly attenuated or lost after wounding. These varied and heterogeneous changes in chemical rCBF regulation following wounding could uncouple brain blood flow from metabolism. This might lead to additional brain damage. cont'd

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT: ☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT. ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION: Unclassified
22a. NAME OF RESPONSIBLE INDIVIDUAL: Mary Frances Bostian	22b. TELEPHONE (Include Area Code): 301-663-7325; 22c. OFFICE SYMBOL: SGRD-RMI-S

DD FORM 1473, 84 MAR — 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE

ıck # 18: After Brain Wounding; Brain stem biogenic Amines After Brain Wounding
ınt'd) The Effect of GM1 Ganglioside, RA 2

ıck # 19: 2) Effect of wounding on selected physiologic variables which might account for
ınt'd) death. In this feline model of brain wounding the immediate cardiorespiratory, "brain stem effects" of missile injury appears stereotyed and similar to that noted in many other species sustaining brain injury. Later occurring, associated intracranial and systemic events of BMW are quite variable, may be species specific and are more often associated with missile injury than closed or percussion head injury. In the present model of brain wounding no one or two physiologic factors appeared to account for the appearance of sustained apnea after wounding. Rather, fatal apnea appeared to result from an interplay of many factors none of which occurred uniformly. These include indirect damage to brain stem respiratory centers from missile energy, loss of CBF autoregulation, CO decrease, cerebral ischemia, ICP, increase or CPP decrease. After BMW death may not occur from an expected apnea but, rather from cardiac arrest.

3) The effect of brain wounding on lung water was insignificant.

4) The effect increased intracranial pressure on brain biogenic amines. The monoaminergic neurotransmitters (EPI, NE, 5-HT) and some their metabolities (DOPAC, HVA, 5-HIAA) were evaluated in selected brain stem areas and the hypothalamus after increases in ICP sufficient to elicit an immediate Cushing response.

In the brain stem areas (LC, area A1C1, NTS and Raphe) large depletions of EPI occurred in all areas. Moderate, although not statistically significant, reductions in NE were also seen in these nuclei. The Raphe nuclei showed indications of reduced serotonergic and dopaminergic functioning. No remarkable effects occurred in the AH, but in the PH both EPI and NE were significantly depleted. We could not determine if the depletions or reductions were due to increased utilization or decrease functioning.

The neurochemical changes consequent to the elevation of ICP and its concomitant Cushing pressor response closely mirror the alterations found in stress studies. Some similarity to stress would be hypothesized because increased ICP causes a massive increase in sympathetic nervous activity leading to a cascade of subsequent sympathetic effects, such as increased plasma catecholamines and increases in MABP.

SUMMARY: This report encompasses 4 separate types of experiments.

1) Chemical regulation of CBF: Normoxic hypocapnia ($PaCO_2$ 21 mmHg) significantly reduced total CBF and many rCBFs both before and after wounding but after wounding this response may have been attenuated except around the wound track where CBF reduction was enhanced. Hyperoxia (PaO_2 > 280 mmHg) hetereogeneously altered rCBF in normal brains: cerebral cortex (-7%) brain stem and cerebellum (+40%). After wounding hyperoxia enhanced some rCBF decreases (-15%) but the flow increases in the brain stem and cerebellum were totally abolished. Hyperoxia plus hypercapnia (PaO_2 > 440 mmHg; $PaCO_2$ ~53 mmHg) dramatically increased CBF in normal brains but this effect was greatly attenuated or lost after wounding. These varied and heterogeneous changes in chemical rCBF regulation following wounding could uncouple brain blood flow from metabolism. This might lead to additional brain damage.

2) Effect of wounding on selected physiologic variables which might account for death. In this feline model of brain wounding the immediate cardiorespiratory, "brain stem effects" of missile injury appears stereotyed and similar to that noted in many other species sustaining brain injury. Later occurring, associated intracranial and systemic events of BMW are quite variable, may be species specific and are more often associated with missile injury than closed or percussion head injury. In the present model of brain wounding no one or two physiologic factors appeared to account for the appearance of sustained apnea after wounding. Rather, fatal apnea appeared to result from an interplay of many factors none of which occurred uniformly. These include indirect damage to brain stem respiratory centers from missile energy, loss of CBF autoregulation, CO decrease, cerebral ischemia, ICP, increase or CPP decrease. After BMW death may not occur from an expected apnea but, rather from cardiac arrest.

3) The effect of brain wounding on lung water was insignificant.

4) The effect increased intracranial pressure on brain biogenic amines. The monoaminergic neurotransmitters (EPI, NE, 5-HT) and some their metabolities (DOPAC, HVA, 5-HIAA) were evaluated in selected brain stem areas and the hypothalamus after increases in ICP sufficient to elicit an immediate Cushing response.

In the brain stem areas (LC, area A1C1, NTS and Raphe) large depletions of EPI occurred in all areas. Moderate, although not statistically significant, reductions in NE were also seen in these nuclei. The Raphe nuclei showed indications of reduced serotonergic and dopaminergic functioning. No remarkable effects occurred in the AH, but in the PH both EPI and NE were significantly depleted. We could not determine if the depletions or reductions were due to increased utilization or decrease functioning.

The neurochemical changes consequent to the elevation of ICP and its concomitant Cushing pressor response closely mirror the alterations found in stress studies. Some similarity to stress would be hypothesized because increased ICP causes a massive increase in sympathetic nervous activity leading to a cascade of subsequent sympathetic effects, such as increased plasma catecholamines and increases in MABP.

FOREWORD:

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National REsearch Council (DHEW Publication No. (NIH) 86-23, Revised 1985).

The experiments on Further Studies on Chemical Control of CBF After Brain Wounding were performed by Dan Torbati, PhD. and June Davidson, B.S.

The experiments on the Effects of Wounding on Selected Physiologic Variables in Spontaneously Breathing, Non-respirated Cats were performed by Dan Torbati, PhD., Alan Jacks, PhD. and June Davidson, B.S.

The experiments on the Effects of Brain Wounding on Lung Water were performed by Dan Torbati, PhD., Alan Jacks, PhD., and June Davidson, B.S.

The experiments on the Effects of Increased Intracranial Pressure on Brain Biogenic Amines were performed by Joseph Soblosky, PhD. and Lynn Rogers, M.D.

The experiments on the Effects of GM_1 Ganglioside and Treatment on Behavioral Recovery After a Missile Wound to the Brain were by Joseph Soblosky, PhD. and J. Bryan Farrell, B.S.

Mrs. Elizabeth P. Hulbert typed this manuscript.

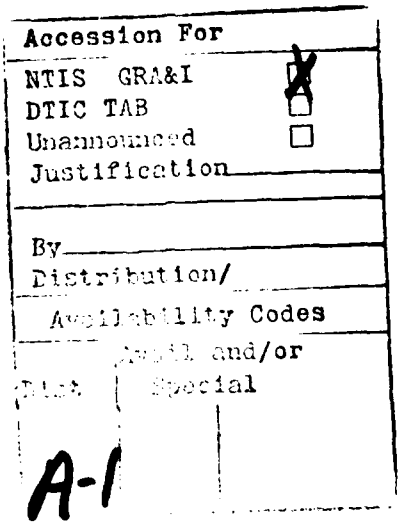


TABLE OF CONTENTS

Page

Section A: Further Studies on Chemical Control of CBF After Brain Wounding

PAGE PURPOSELY BLANK

SECTION A: FURTHER STUDIES ON CHEMICAL CONTROL OF CBF AFTER BRAIN WOUNDING

INTRODUCTION

Regulation of cerebral blood flow (CBF) is a complex process involving several mechanisms: mechanical (whereby CBF remains constant over wide ranges of systemic arterial and intracranial pressures), chemical (in which CBF responds to chagnes in arterial CO_2 and O_2 levels), metabolic (whereby CBF is altered to meet regional cerebral energy demainds) and neurogenic (whereby sympathetic and parasympathetic nervous control may affect CBF); (for bibliography see previous annual report DAMD17-86-C-6098, April 1989).

How the intact brain controls its blood supply is of fundamental importance and knowledge of how these control mechanisms may be deranged after brain wounding is of interest not only from a theoretic point of view but from a practical one as well because therapy designed to improve brain function after injury may depend upon intact CBF regulatory mechanisms.

In our prior yearly report we presented data on mechanical CBF regulation and some aspects of chemical regulation of CBF following missile injury. The effect of wounding on chemical regulation of regional CBF (rCBF) was tested by determining the effect of hypoxemia and hypercapnia on rCBF both before and after wounding. The experiments demonstrated that after a brain missile wound both mechanical (autoregulation) and chemical (hypoxemia, hypercapnia) control of CBF were widely impaired throughout the brain.

Because control of CBF is so critical for brain function, from 4/88 to 4/89 we continued evaluating post-wounding chemical regulation of rCBF by measuring responses to hypocapnia and hyperoxia. Normally, both of these conditions decrease CBF (11,26,33-34). How missile wounding alters these responses has never before been determined and experiments to elucidate these points have physiologic and clinical relevance:

1. Hyperventilation-induced hypocapnia is widely used to rapidly treat increased intracranial pressure (ICP) (1,11,30,32,37,38,40,46,53). Hypocapnia decreases CBF and cerebral blood volume in normal brains and thereby reduces elevated ICP by shrinking the "blood compartment". Since our prior work has shown that a brain missile wound (BMW) reduces or abolishes CBF responsiveness to chemical stimuli, we felt it of paramount importance to test whether the missile-wounded brain responds to decreased $PaCO_2$ by decreasing CBF. If so, hyperventilation might be expected to be a worthwhile maneuver in attempting to reduce elevated ICP in a brain-wounded individual. If not, hyperventilation-induced hypocapnia might be expected to be of no use in the treatment of elevated ICP following a brain wound. The purpose of these experiments was to determine the effect of a short term hyperventilation-induced hypocapnia on rCBF following brain wounding.

2. Clinically, increased concentrations of normobaric oxygen may be used in breathing mixtures to improve cerebral oxygenation should hypoxemia occur following brain injury. It is well known that increasing normobaric

arterial pO_2 (hyperoxia) decreases CBF (32-34) The higher level of arterial pO_2, however, keeps the brain well oxygenated (2,39) despite the decreased blood flow. Since gas mixtures providing oxygen concentrations above 20% could be used to treat or resuscitate those with brain injury, the purpose of these experiments was to ascertain how increasing arterial pO_2 affects rCBF in the wounded brain and specifically to know whether after BMW, vasoconstriction induced by hyperoxia might lead to dangerously low rCBFs.

3. If the brain is exposed to a hypoxic insult (as with airway obstruction) the associated hypercapnia is beneficial because the elevated $PaCO_2$ increases CBF and may improve brain oxygenation. Our prior studies have shown, however, that after a missile wound to the brain neither hypercarbia alone nor hypoxemia by itself produce the normally expected increase in CBF. In fact, these stimuli even decrease blood flow around the wound track within the brain.

Experimentally, giving a gas mixture of increased O_2 and CO_2 concentrations leads to a pronounced cerebral vasodilitation and increased cerebral oxygenation (2). How this gas combination would affect the missile-wounded brain is unknown. If the vasodilatory effect of hypercapnia were lost, and even if CBF decreased slightly because of the hyperoxia, a several fold increase in PaO_2 should increase regional tissue PO_2 and promote better brain function. The purpose of these experiments, therefore, was to evaluate the effect of a hyperoxic-hypercarbic gas mixture on rCBF to see if this gas combination should be considered as a therapy for the missile-wounded brain.

THUS, DATA IN SECTION A OF THIS YEARLY REPORT EXAMINE HOW A BRAIN MISSILE WOUND ALTERS THE BRAIN'S ABILITY TO REGULATE CBF UNDER CONDITIONS OF 1) HYPOCAPNIA WITH A NORMAL PaO_2 (NORMOXIC-HYPOCAPNIA) 2) HYPEROXIA WITH NORMAL $PaCO_2$ (HYPEROXIC-NORMOCAPNIA) AND 3) HYPEROXIA WITH ELEVATED $PaCO_2$ (HYPEROXIC-HYPERCAPNIA).

GENERAL METHODS

Surgical Procedures: Mongrel cats of either sex (2.5 to 5 kg) were anesthetized with 30-40 mg/kg pentobarbital i.p. After endotracheal intubation, one femoral artery was cannulated (PE 160) for blood pressure recording and blood sampling for measurements of PaO_2, $PaCO_2$, and hematocrit (HCT). The second femoral artery was used for placement of an intracardiac PE 90 pigtail catheter for microsphere injection. Accurate catheter tip placement was determined by ventricular pressure recordings and post mortem examination. A femoral vein was also cannulated (PE 90) for additional administration of pentobarbital, as needed, during surgery and gallamine for paralysis in studies of chemical CBF regulation. Both brachial arteries were cannulated (PE50) to withdraw arterial blood samples for microsphere counting (reference sample method) (see previous annual report).

The cat was then placed in a stereotaxic head apparatus, a midline scalp incision was made, and the anterior wall of the right frontal sinus removed prior to wounding (7). Three miniature, stainless steel screws connected to shielded wires were used for electroencephalographic (EEG) recording. One screw was placed in the skull over the right parietal cortex and two over the left. A 4 mm diameter left parietal burr hole was made for intracerebral insertion of a fiberoptic ICP recording probe (Camino, model 420). The EEG screws and the ICP probe were then secured and sealed with methylmethacrylate. Two needle electrodes were placed subcutaneously over the left thoracic cage about 2 or 3 cm anteriolateral to the sternum for electrocardiographic (ECG) recording. After all surgical preparations the cats were again evaluated for a satisfactory depth of anesthesia. Cats used to study chemical control of CBF were paralyzed with 30-40 mg of i.v. gallamine and immediately placed on a respirator.

SPECIFIC METHODS FOR FURTHER STUDIES ON CHEMICAL CONTROL OF CBF

1. The effect of normoxic-hypocapnia on rCBF following a brain wound; (Group I, N=10): After completion of surgery and placement of the cat in the stereotaxic frame, a microsphere injection was made for a control rCBF measurement when the cats were both normoxic and normocapnic. Approximately 10 min later, arterial hypocapnia was induced by 10 minutes of hyperventilation. This reduced $PaCO_2$ by about 10 mmHg to 21 mmHg. At this time a second rCBF was measured to determine the normal responsiveness of the cat's cerebral vessels to hypocapnia. After a 30 min recovery period we verified that the animals were normoxic and normocapnic by a blood gas measurement. The cats were then wounded by a 2 mm diameter, 31.7 mg steel sphere in the right cerebral hemisphere (7). Missile energies averaged 1.4 Joules. The time of wounding was designated as "zero time". Thirty min after the brain missile wound, a third rCBF was measured when the cats were in a normoxic and normocapnic state to determine baseline rCBF values after wounding. Post-BMW hypocapnia was then induced by hyperventilation for 10 min. This again reduced arterial $PaCO_2$ to a mean of about 21 mmHg. The fourth rCBF was measured during this ongoing hyperventilation, 45 min post-BMW. After completion of all blood flow measurements the cats were painlessly sacrificed by an overdose of i.v. pentobarbital. Tissue fixation, brain dissection and microsphere counting were performed as described in our previous annual report (Annual Report DAMD17-86-C-6098; April, 1989).

2. The effect of hyperoxia on rCBF; following a brain wound; (Group II, N=9). The experimental design was exactly as described for Group I, except that hyperventilation was replaced by 100% O_2 breathing for 10 min both before and after wounding. Breathing 100% O_2 raised the PaO_2 to greater than 280 mmHg in each instance. These cats remained isocapnic, $PaCO_2$ 26 to 30 mmHg. In 3 cats in this group, thirty minutes after the post-BMW hyperoxic trial had been discontinuted and arterial blood gases had returned to normoxic, normocapnic levels, CBF was measured for the 5th time to examine whether rCBFs in the wounded brain could return to post-wounding baselines following the hyperoxic challenge.

3. The effect of simultaneous hypercapnia and hyperoxia on rCBF (Group III, N=3). The experimental design was similar to that of Group I, except that hyperventilation was replaced by breathing a mixture* of 95% O_2 and 5% CO_2 causing the interval PaO_2 to be greater than 400 mmHg and the interval $PaCO_2$ to be about 53 mmHg.

STATISTICAL ANALYSIS All data are expressed as mean ± SE. In groups 1 to 3, control values for CBF and other physiologic variables were compared with their corresponding values during either a hypocapnic, hyperoxic or hypercapnic- hyperoxic trial before wounding and after wounding. The variances within the same group were first evaluated by analysis of variance; the differences were then tested by the paired t-test. The degree of rCBF responsiveness to chemical stimuli before brain wounding was also compred to the rCBF responsiveness to chemical stimuli after wounding. The before wounding responsiveness (r_1) is defined as the absolute baseline rCBF value minus the rCBF value in response to the chemical stimulus. The post wounding responsiveness (r_2) equals the post wounding baseline rCBF minus the post wounding rCBF value in response to the chemical stimulus. For this comparison analysis of varience was followed by a modified t-test with Bonferroni correction. The unpaired t-test was also used to compare means of variables between different groups. Significance was determined by a $p < 0.05$.

* between experiments 2 and 3 we changed blood gas machines from an Instrumentation Laboratory to a Radiometer machine. The highest PaO_2 reading possible on the IL machine was 280 mmHg while that on the Radiometer is 800 mmHg. In experiment A_2 the PaO_2 levels of animals exposed to hyperoxia approximated 400 mmHg but this could not be recorded by the IL instrument.

RESULTS

1) THE EFFECT OF NORMOXIC HYPOCAPNIA ON rCBF FOLLOWING A BRAIN WOUND; (GROUP I N=10)

We had 5 successful experiments where the effects of hypocapnia on CBF could be determined both before and after BMW (Table 1). Five additional cats in this group developed either an extremely reduced or flat EEG within 30 min after wounding. Their total post-wounding, normocapnic CBF was only about 11 ml/100g/min which fell to 6 ml/100g/min during hypocapnia. Brains in these cats were severely ischemic and their post-BMW CBF data are excluded from analysis. Their pre-BMW hypocapnic response was valid, however, and these data (N=8 to 10) were used to enhance our evaluation of the normal feline cerebral vascular response to hypocapnia.

a) Cerebral Blood Flows Before Brain Wounding

Reduction of $PaCO_2$ from 31 to 21 mmHg by hyperventilation significantly reduced total CBF from 31.3 to 25.6 ml/100g/min, (-18%). Various degrees of reduction in rCBF were observed throughout the brain. Cerebral cortex and cerebellum appeared most affected with flow reductions ranging from 15% to 22%. White matter in the cerebral hemisphere exhibited a 14% to 19% decrease in flow while the caudate nucleus demonstrated a 14% fall. The thalamus had the largest flow decrease (24%) while the reticular formation fell the least (7%). These decreases were statistically significant in 2 of 14 brain areas: the parietal cortex and thalamus (Figure 1). If one includes the prewound data from the experiments which failed after wounding, however, 9 of 14 sampled brain structures exhibited significant rCBF decreases as a result of the hypocapnia, (Table 2-A).

b) Cerebral Blood Flows After Brain Wounding

After brain wounding in the normoxic, normocapnic condition, total CBF fell from 31 to 23 ml/100g/min probably because of autoregulation loss and increased ICP. The response to the superimposed hypocapnic challenge was attenuated after BMW because when $PaCO_2$ was again lowered to 21 mmHg, total CBF fell from 23 ml/100g/min to only 20 ml/100g/min, an 11% reduction. The reduction in total CBF was significant as were rCBF reductions in 6 separate structures including periwound white and periwound gray matter unwounded white matter, caudate nucleus, thalamus and frontal cortex (Table 1, Figure 1).

In contrast to the rest of the brain, damaged brain about the wound track exhibited an increased response to hypocapnea. Before wounding, brain in the region of the missile track exhibited a 14% to 18% rCBF reduction when $PaCO_2$ was reduced to 21 mmHg but afterwards a similar $PaCO_2$ reduction caused periwound rCBFs to decrease 24% to 27%, an enhanced effect. (Figure 1)

The responsiveness to hypocapnia before injury (r_1) as compared to post-BMW responsiveness (r_2) showed no significant difference in most structures. For example: prior to wounding hypocapnia caused a 19% flow reduction in the white matter while afterwards it was 18%; the $PaCO_2$ reductions caused reticular formation blood flow to decrease 7% both before and after wounding. After wounding, cerebellar rCBFs showed no change at all to the hypocapnic challenge indicating that these vessels had totally lost their vasoconstrictive response to lowered $PaCO_2$.

c) The Response of Other Physiologic Variables to Hypocapnia Before and After Brain Wounding

The various physiologic variables measured during the hypocapnic experiments are presented in Table 2. Cats in this group were normotensive during the entire experimental period both before and after wounding (MABP 110-128 mmHg). Control ICP was 11 mmHg. During the control period hypocapnia reduced CPP from 117 mmHg to 107 mmHg (6%) owing to a decrease in MABP. Brain wounding induced a rapid static increase in ICP to 53 mmHg which was still present 30 to 45 minutes after wounding when the post BMW rCBFs during normocapnic and hypocapnic periods were measured. After wounding, hyperventilation also caused a fall in CPP from 64 mmHg to 58 mmHg (9%) again by decreasing MABP. FOLLOWING BRAIN WOUNDING 10 MIN OF HYPERVENTILATION DID NOT ALTER THE ELEVATED ICP: EVEN THOUGH THE $PaCO_2$ WAS REDUCED TO 21 mmHg, ICP REMAINED AT 53 mmHg. THESE DATA SUGGEST THAT IF MISSILE WOUNDING OF THE BRAIN IS ASSOCIATED WITH A GREATLY INCREASED ICP, A SHORT PERIOD OF HYPERVENTILATION MAY NOT LOWER ICP EVEN THOUGH IT MAY ACHIEVE A MODEST REDUCTION IN CBF.

Before BMW, hypocapnia ($PaCO_2$ 21 mmHg) reduced significantly H^+ from 47 nmol/L to 37 nmol/L (21%). After wounding, arterial H^+ increased to 49 nmol/L despite a normal $PaCO_2$ indicating a slight post wounding arterial acidosis. Hypocapnia then effected an arterial H^+ reduction to 39 nmol/L, a 21% decrease.

Table 1: Changes in rCBF in response to hypocapnia before and after BMW in completed experiments. Pre-wound hypocapnic flows are compared to pre-wound normocapnic flows. Post-wound hypocapnic flows are compared to post-wound normocapnic flows. *Significant; $p<0.05$. Mean $\pm$ SE, (ml/100g/min); n=5.

	PRE-WOUND		POST-WOUND	
VARIABLES	NORMOCAPNIA	HYPOCAPNIA	NORMOCAPNIA	HYPOCAPNIA
PaCO2 (mmHg)	31.0	21.4	32.2	21.0
FRONTAL CORTEX	36.28 5.12	28.70 2.43	25.64 3.63	21.16* 3.04
PARIETAL CORTEX	33.31 3.43	25.86* 2.67	23.04 3.06	21.22 3.18
TEMPORAL CORTEX	24.94 2.47	19.40 1.44	21.16 2.53	19.26 2.47
OCCIPITAL CORTEX	35.02 3.62	29.72 3.11	23.70 3.37	22.36 3.72
PERIWOUND GRAY	29.42 3.20	25.06 3.06	24.08 3.02	18.18* 2.09
PERIWOUND WHITE	31.18 3.80	26.94 3.40	30.40 2.82	22.22* 3.22
WHITE MATTER	26.12 1.53	21.34 1.94	20.40 1.11	16.74* 1.43
CAUDATE NUCLEUS	43.76 3.95	37.82 4.34	33.70 6.26	27.28* 4.71
HIPPOCAMPUS	21.46 1.55	17.88 2.18	17.54 1.59	15.98 1.03
THALAMUS	39.18 3.67	29.78* 1.33	27.52 2.00	23.16* 2.11
RETICULAR FORMATION	32.14 3.43	29.90 3.74	23.90 2.85	22.32 3.13
BRAIN STEM & MEDULLA	29.90 1.47	25.72 2.19	21.60 1.19	20.56 2.01
CEREBELLUM-GRAY	42.24 4.96	33.14 4.11	25.20 3.11	25.36 3.85
CEREBELLUM-WHITE	41.82 2.97	34.16 3.87	27.34 2.51	27.00 2.79
TOTAL CBF	31.30 2.45	25.60 1.89	23.10 2.09	20.46* 2.29
SPINAL CORD	14.14 1.48	12.58 1.52	11.90 1.55	11.42 1.50

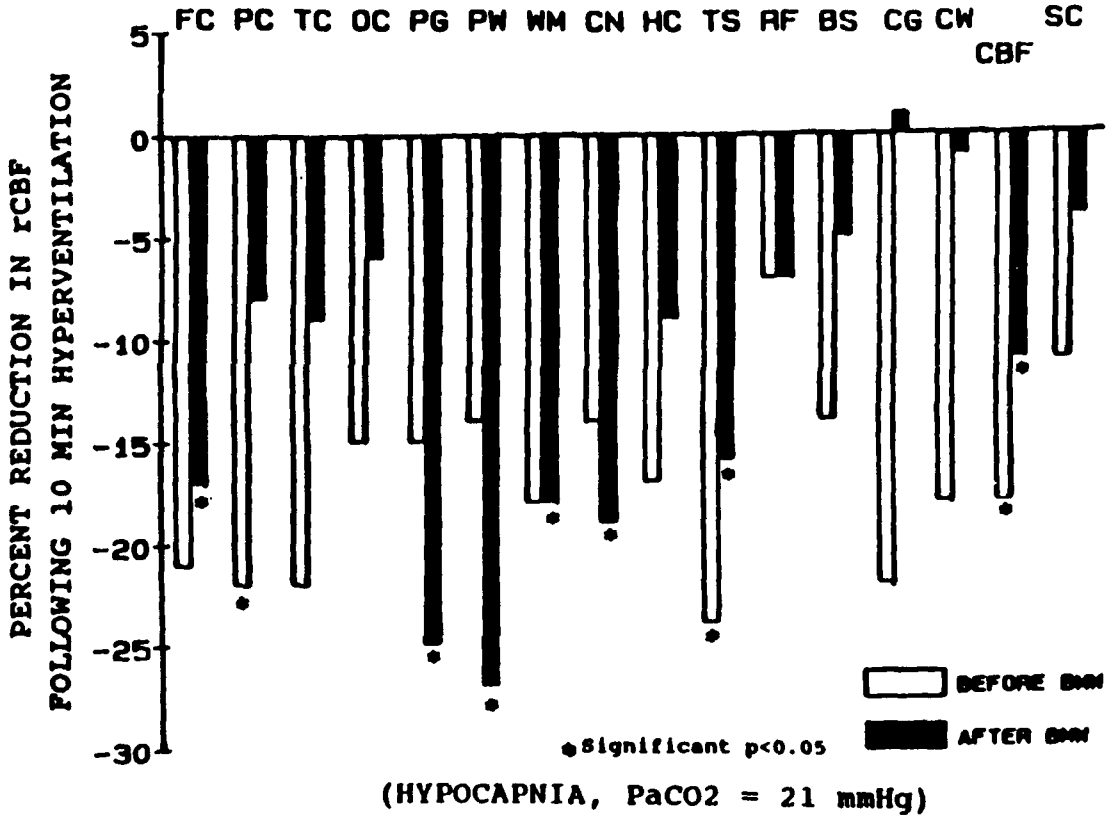


Figure 1: Percent change in rCBF following 10 min hyperventilation to produce hypocapnia before and after BMW. Before wounding, hypocapnia produced 15% to 25% decrease in the rCBF of all structures. After wounding, hypocapnia produced a slightly enhanced vasoconstriction in periwound structures, attenuated the vasoconstrictive response in the brain stem and cerebellum and preserved the reactivity in cortical and limbic structures. FC, PC, TC and OC=frontal, parietal, temporal, and occipital cortecies; PG and PW=periwound gray and white matter; WM=white matter; CN=caudate nucleus; HC=hippocampus; TS=thalamus; RF=reticular formation; BS=brain stem; CG and CW=cerebellum gray and white matter, and SC=spinal cord.

Table 2: Changes in physiological variables in response to hypocapnia before and after BMW in completed experiments. Pre-wound hypocapnic and normocapnic variables are compared; post-wound hypocapnic and normocapnic variables are compared. *Significant; $p<0.05$. (Mean $\pm$ SE); n=5.

	PRE-WOUND		POST-WOUND	
VARIABLES	NORMOCAPNIA	HYPOCAPNIA	NORMOCAPNIA	HYPOCAPNIA
pH	7.33 0.03	7.44* 0.04	7.29 0.03	7.42* 0.04
[H+] (nmol/l)	47.18 3.24	36.92* 3.82	52.06 4.61	38.52* 3.45
PaO2 (mmHg)	106.20 5.54	121.80 6.55	106.00 6.24	118.60 8.74
PaCO2 (mmHg)	30.98 1.02	21.36* 1.56	32.16 1.91	21.04* 1.42
MABP (mmHg)	128.40 10.75	117.60 8.07	116.60 8.87	110.80 5.45
ICP (mmHg)	12.00 2.86	11.00 2.02	53.00 7.66	53.20 11.99
CPP (mmHg)	116.60 11.28	106.80 8.64	63.80 14.74	57.60 16.36
CVR (CPP/CBF)	3.95 0.63	4.34 0.56	2.78 0.60	2.74 0.68
HEART RATE/min	207.00 16.55	205.00 17.75	195.00 17.75	208.00 15.94
HEMATOCRIT (%)	30.00 4.30	29.60 2.68	32.60 2.62	30.80 2.69

Table 2A: Pre-wounding rCBF(ml/100g/min) during normoxic-normocapnia and normoxic-hypocapnia in 10 cats; (Mean ± SE). Total CBF, as well as 9/14 brain structures showed significant decreases in rCBF during hypocapnia (Paried t-test; n=8 for Cortices).

STRUCTURE	NORMOCAPNIA	HYPOCAPNIA
PaCO2 (mmHg)	31.4	20.3
FRONTAL CORTEX	40.00 5.47	31.75* 3.73
PARIETAL CORTEX	39.13 4.94	31.38* 4.66
OCCIPITAL CORTEX	43.12 4.91	34.87* 3.92
TEMPORAL CORTEX	28.38 4.71	24.00 4.01
WHITE MATTER	35.10 5.26	26.00* 2.46
PERIWOUND WHITE	41.10 5.67	33.40* 4.12
PERIWOUND GRAY	34.25 6.28	29.00* 4.81
BRAIN STEM	37.80 4.98	30.30 2.33
CEREBELLUM GRAY	49.30 7.95	36.50 3.78
CEREBELLUM WHITE	43.38 5.33	38.50 5.03
HIPPOCAMPUS	27.60 3.80	21.20* 2.68
THALAMUS	52.10 8.73	38.10* 4.86
RETICULAR FORMATION	40.40 6.06	33.60 3.47
CAUDATE NUCLEUS	57.80 6.02	43.80* 6.19
TOTAL CBF	40.59 4.38	31.35* 2.95

2) THE EFFECT OF HYPEROXIA ON rCBF FOLLOWING A BRAIN WOUND; (GROUP II N=9)

Four of 9 cats in this group became brain dead within 30 minutes of wounding owing to post wounding ICPs of ~ 95 mmHg and CPPs of ~ 10 mmHg. After injury these 4 cats exhibited reduced or flat EEGs and had CBFs ranging from 0 to 10 ml/100gm/min. The post wounding data from such animals can provide no information on the effect of hyperoxia on CBF after wounding but their prewound rCBF changes to increased PaO_2 may be used to enhance the assessment of the normal feline cerebral blood flow response to hyperoxia.

a) Cerebral Blood Flows Before Brain Wounding

Analysis of the 5 successful experiments (Table 3) reveals that total CBF in normal brain was not changed by the hyperoxic challenge being ~ 32 ml/100g/min when arterial pO_2 was 129 mmHg and ~34 ml/100g/min when arterial pO_2 was increased to >280 mmHg. Analysis of rCBFs, however, revealed 3 distinct rCBF patterns in response to hyperoxia: 1) cerebral cortex and white matter, hippocampus, and caudate nucleus had ~5% to 15% decrease in flow; 2) the thalamus exhibited no change in flow; 3) the brain stem, medulla and cerebellum had flow increases of 26% to 45% after hyperoxia (Figure 2). Inclusion of pre-wound data from the 4 cats that died after wounding allow these changes to reach statistical significance, ($p<0.025$, paired t-test), table 4-A.

b) Cerebral Blood Flows After Brain Wounding

The post wounding CBF response to hyperoxia could be determined in only 5 cats but the rCBF trends appeared markedly different from the unwounded situation. Baseline total CBF averaged 19.5 ml/100g/min in these cats following BMW probably because post wounding ICPs averaged ~64 mmHg and autoregulation was impaired. The imposition of hyperoxia on the wounded brain slightly but significantly reduced total CBF to 17.0 ml/100g/min. After wounding, hyperoxia appeared to enhance rCBF reductions in several brain structures. For instance, prior to wounding the hyperoxic challenge decreased cortical rCBFs 5% to 10%. After wounding, hyperoxia caused cortical flow decreases of 12% to 21%. Flow reductions in the damaged, periwound tissues were also increased secondary to hyperoxia: before wounding, hyperoxia caused flow decreases of 11% to 15% in brain tissue destined to be adjacent to the missile track. After brain injury, periwound gray and white matter flows diminished 31% to 34%. Whereas prior to wounding the brainstem- medulla and cerebellum showed very large CBF increases to elevated PaO_2, after wounding these same brain areas showed flow decreases similar to the rest of the brain when PaO_2 was raised.

The ability of rCBFs to recover from a hyperoxic challenge after brain wounding was evaluated in 3 cats (Table 4). Total CBF after brain wounding averaged ~21 ml/100g/min in these animals. With the hyperoxic challenge total CBF fell to ~19 ml/100g/min but 30 minutes after cessation of hyperoxia total CBF was again ~21 ml/100g/min. Most rCBFs except those about the wound track also returned to baseline levels with discontinuance of hyperoxia. Periwound tissues behaved differently to the hyperoxic

challenge after wounding: their blood flow averaged ~32 ml/100g/min after wounding when PaO_2 was normal. Following the post wounding hyperoxic stimulus (PaO_2 at 133 mmHg), flows decreased to 22 ml/100g/min (~30% diminution). With cessation of the postwounding hyperoxic challenge periwound rCBF fell even further to ~19 ml/100g/min (~40% flow decrease). In other words the periwound tissues did not recover like the rest of the undamaged brain once PaO_2 was returned to normal levels.

c) The Response of Other Physiologic Variables to Hyperoxia Before and After Brain Wounding.

The various physiologic variables measured during the hyperoxic experiments are presented in Table 5. Before brain wounding ICP approximated 15 mmHg; MABPs were about 140 mmHg and CPPs, therefore, ~125 mmHg. The hyperoxic challenge tended to increase MABP and ICP slightly and CPP rose from 119 mmHg to 130 mmHg. H^+ remained between 43 nmol to 45 nmol/l both before and after the hyperoxic challenge.

After brain wounding when the animals were normoxic the ICPs averaged 64 mmHg. The mean MABP fell slightly to 114 mmHg and mean CPP, therefore, was 50 mmHg. Following the post wounding hyperoxic challenge, CPP fell to 40 mmHg owing primarily to a reduction in MABP to ~100 mmHg. H^+ remained 45 nmol/l to 48 nmol/l.

Table 3: Changes in rCBF in response to hyperoxia (100% O_2 breathing) before and after BMW in 5 completed experiments. Pre-wound hyperoxia and compared to pre-wound normoxia; post-wound hyperoxia compared to post-wound normoxia. *Significant; $p<0.05$. Mean ± SE, (ml/100g/min).

	PRE-WOUND		POST-WOUND	
VARIABLES	NORMOXIA	HYPEROXIA	NORMOXIA	HYPEROXIA
PaO2 (mmHg)	129.0	280.0	128.0	280.0
FRONTAL CORTEX	39.20 9.65	36.20 9.84	19.40 2.68	16.80 3.02
PARIETAL CORTEX	44.40 12.23	42.20 13.44	18.80 2.56	16.60 3.94
TEMPORAL CORTEX	28.20 8.01	25.80 5.32	16.80 2.13	14.20 2.11
OCCIPITAL CORTEX	55.40 15.30	49.80 14.30	21.80 5.18	17.20 4.66
PERIWOUND GRAY	37.60 7.90	31.80 6.40	26.20 5.97	17.20 3.26
PERIWOUND WHITE	35.60 7.49	31.40 6.76	27.20 7.96	18.80 4.85
WHITE MATTER	30.80 5.44	28.60 6.98	16.40 1.40	14.80 2.84
CAUDATE NUCLEUS	35.50 4.97	31.75 5.45	24.75 2.78	21.25 2.87
HIPPOCAMPUS	17.60 2.36	17.20 2.63	12.80 1.53	10.20 1.66
THALAMUS	34.40 5.10	32.00 5.62	21.40 3.54	20.20 3.31
HYPOTHALAMUS	13.00 1.52	14.20 3.26	8.60 0.51	6.40 0.98
RETICULAR FORMATION	31.00 4.37	39.20 11.31	20.00 4.16	18.40 4.33
BRAIN STEM &MEDULLA	24.40 4.37	34.00 8.45	17.60 4.33	15.80 4.71
CEREBELLUM GRAY	34.00 7.62	49.40 15.90	20.60 3.12	21.40 4.21
CEREBELLUM WHITE	39.20 8.31	52.40 17.56	26.60 5.05	23.20 5.14
TOTAL CBF	32.26 6.09	33.78 7.31	19.52 2.47	16.96* 3.05
SPINAL CORD	13.80 2.13	18.40 2.73	14.40 2.58	17.60 6.80

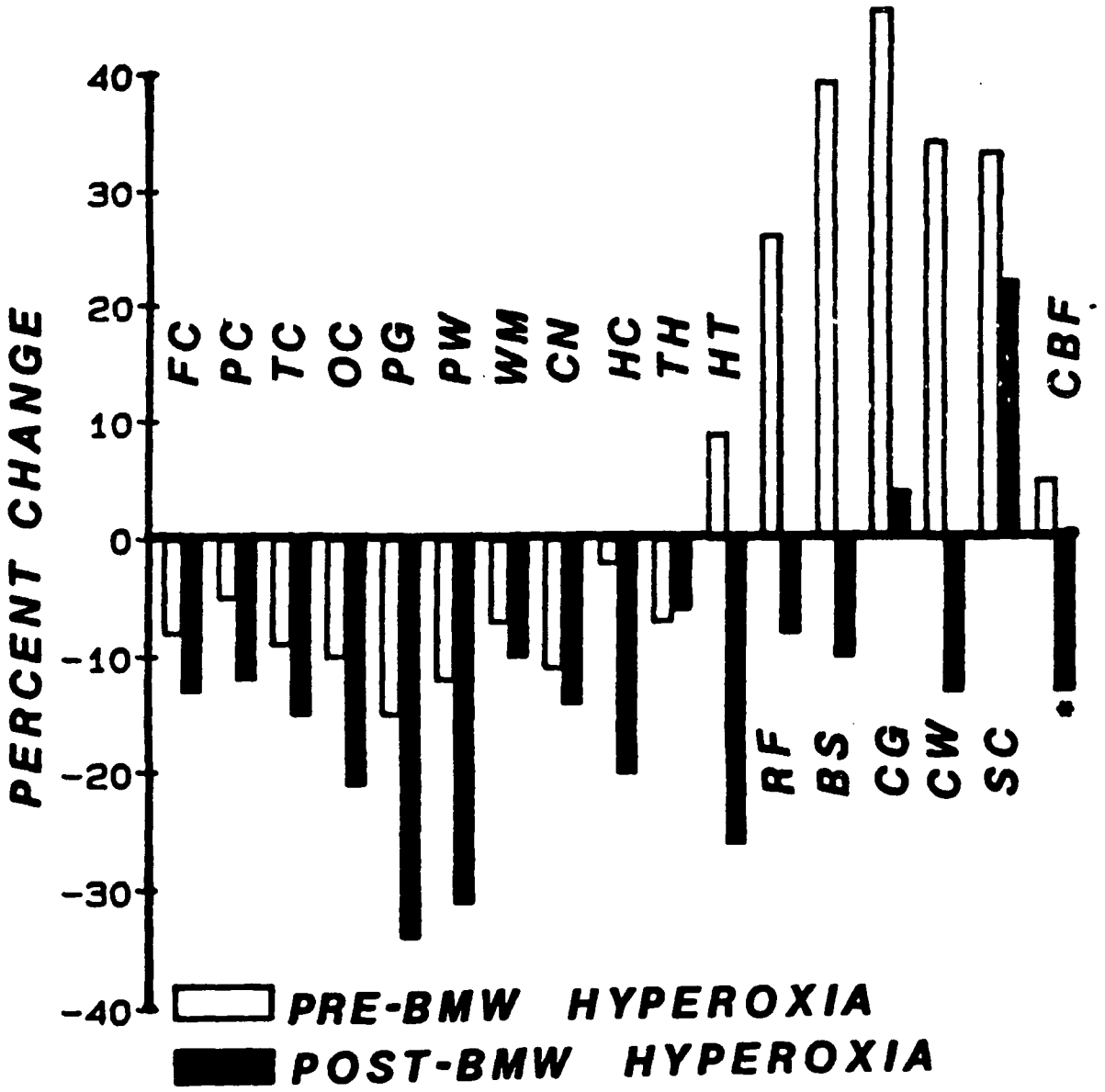


Figure 2: Percent change in rCBF following 10 min hyperoxia (100% oxygen breathing) before and after BMW. Before wounding, hyperoxia produced slight decreases in rCBF of cortical and subcortical structures and large increases in rCBF in the cerebellum, brain stem, medulla and reticular formation. After wounding, the vasoconstrictive effect of hyperoxia was relatively enhanced particularly in the periwound structures, and the pre-BMW vasodilatory effect of hyperoxia was attenuated. These effects led to a *significant reduction in total CBF after wounding. Blood flow to the spinal cord was not affected during post-wounding hyperoxia. Legends for brain structures, see Fig.1.

Table 4: Changes in post-BMW rCBF during normoxic air breathing before and after 10 min hyperoxic trail (100% O_2 breathing). Thirty min after hyperoxic test, the baseline values were completely recovered in all structures, except for periwound gray and white matter in which 40-42% decrease in rCBF was still present. Mean ± SE, (ml/100g/min), (n=3)

		POST-WOUND	
VARIABLES	NORMOXIA	HYPEROXIA	NORMOXIA
FRONTAL CORTEX	18.67 4.81	17.33 3.79	21.33 5.36
PARIETAL CORTEX	19.33 4.48	18.00 4.97	21.33 7.33
TEMPORAL CORTEX	15.00 3.21	14.67 2.62	17.33 4.37
OCCIPITAL CORTEX	26.00 8.08	21.33 5.04	28.33 11.57
PERIWOUND GRAY	32.00 8.72	21.00 2.94	18.67 2.91
PERIWOUND WHITE	31.67 13.12	23.67 4.90	19.33 4.18
WHITE MATTER	17.33 2.33	17.33 3.06	19.33 5.36
CAUDATE NUCLEUS	26.00 3.51	22.67 2.49	28.00 4.36
HIPPOCAMPUS	14.33 2.19	11.33 1.93	12.33 2.73
THALAMUS	25.00 4.51	24.67 1.93	23.33 3.18
HYPOTHALAMUS	9.00 0.58	7.67 0.47	10.33 2.03
RETICULAR FORMATION	24.67 5.24	23.33 3.17	21.67 4.70
BRAIN STEM & MEDULLA	22.67 4.70	21.33 3.79	21.33 5.46
CEREBELLUM GRAY	22.67 4.81	24.67 3.68	23.00 5.86
CEREBELLUM WHITE	31.33 7.54	29.33 4.37	28.00 6.81
TOTAL CBF	20.83 3.76	19.23 3.21	20.73 5.20

Table 4A: Pre-wounding rCBF(ml/100g/min; Mean ± SE) during normoxia and hyperoxia in 9 cats. Brain stem and cerebellum showed significant increases in rCBF during hyperoxia (Paired t-test).

STRUCTURE PaO2 (mmHg)	NORMOXIA 130.0	HYPEROXIA 280.0
FRONTAL CORTEX	34.44 5.58	33.11 5.42
PARIETAL CORTEX	37.88 7.00	38.66 7.39
OCCIPITAL CORTEX	49.00 8.83	49.11 8.76
TEMPORAL CORTEX	25.44 4.47	24.77 2.99
WHITE MATTER	28.33 3.17	26.77 3.79
PERIWOUND WHITE	33.11 4.07	30.66 3.61
PERIWOUND GRAY	37.77 4.45	36.66 4.53
BRAIN STEM	24.55 2.53	32.11* 4.66
CEREBELLUM GRAY	32.77 4.46	48.00* 9.54
CEREBELLUM WHITE	36.33 4.62	46.55 9.66
HIPPOCAMPUS	19.00 1.87	18.33 1.64
THALAMUS	34.55 2.88	35.44 4.02
RETICULAR FORMATION	33.55 2.90	39.44 6.18
CAUDATE NUCLEUS	40.44 3.90	42.55 7.75
TOTAL CBF	30.44 3.38	32.50 3.78

Table 5: Changes in physiological parameters in response to hyperoxia before and after BMW in completed experiments. Pre-wound hyperoxic and normoxic variables are compared; post-wound hyperoxic and normoxic variables are compared. *Significant; $p<0.05$. (Mean ± SE), (n=5)

	PRE-WOUND		POST-WOUND	
VARIABLES	NORMOXIA	HYPEROXIA	NORMOXIA	HYPEROXIA
pH	7.34 0.02	7.37 0.02	7.32 0.02	7.35 0.02
[H+] (nmol/l)	45.74 2.70	43.32 2.49	48.00 1.77	45.48 2.35
PaO2 (mmHg)	128.78 9.02	280.00* 0.00	128.42 16.58	280.00* 0.00
PaCO2 (mmHg)	30.06 1.38	28.10 2.03	27.52 0.76	26.20 1.13
MABP (mmHg)	143.00 28.03	138.80 29.02	114.20 11.36	99.60 8.41
ICP (mmHg)	14.75 3.50	13.75 3.54	64.00 7.71	60.00 7.36
CPP (mmHg)	110.00 29.67	105.75 30.29	48.00 18.52	42.00 16.70
CVR (CPP/CBF)	4.03 1.13	3.90 1.18	2.30 0.93	2.40 1.13
HEART RATE/min	195.00 8.22	192.00 13.75	198.00 5.61	195.00 9.49
CARDIAC BLOOD FLOW (ml/100g/min)	181.40 31.91	203.80 39.80	186.40 29.00	166.00 26.58
CARDIAC OUTPUT (ml/min)	255.20 48.75	225.50 39.63	264.60 52.77	214.60 35.22
HEMATOCRIT (%)	34.00 4.57	35.00 4.62	37.50 0.52	32.20 3.26

3) THE EFFECT OF HYPEROXIC-HYPERCAPNIA ON rCBF FOLLOWING A BRAIN WOUND; (GROUP III, N=3)

a) Cerebral Blood Flows Before Brain Wounding

In normal, unwounded cats total CBF rose dramatically and highly significantly in response to the hyperoxic hypercapnic stimulus: from 35 to 83 ml/100g/min. Regional CBFs all increased; the increases were significant in 11/14 structures, (Table 6).

b) Regional Cerebral Blood Flows After Brain Wounding

After wounding the increase in CBF because of hyperoxia plus hypercapnia was severely attenuated or abolished. The baseline total CBF after wounding was 27 mmHg; Following the hyperoxic-hypercapnic stimulus total CBF showed no significant change. Most telencephalic brain areas sampled showed only slight increases in rCBF from 6% to 18%, greatly attenuated compared to the prewound hyperoxic-hypercarbic increase (Figure 3,). Cerebellar rCBF showed the largest post wounding rise to hyperoxia-hypercarbia: before this chemical stimulus, cerebellar blood flow averaged ~31 ml/100g/min; afterwards, cerebellar rCBF rose significantly (23%) to 38 ml/100g/min. This amount of rCBF rise, though substantial, was greatly reduced from that observed before wounding. Periwound tissues showed diminished flow to the hyperoxic-hypercarbic challenge. After wounding periwound gray had a baseline flow of 25 ml/100g/min while the periwound white flow was 27ml/100g/min With the increase in $PaCO_2$ to 54 mmHg and the PaO_2 to >400 mmHg these rCBFs fell to 18 and 19 ml/100g/min respectively, this latter decrease being significant. Overall the post-BMW responsiveness to the hyperoxic-hypercarbic challenge was significantly reduced in 11/14 structures.

c) The Response of Other Physiologic Variables to Simultaneous Hyperoxia and Hypercapnia Before and After Brain Wounding

In this group, control pre wound arterial pCO_2 was 30 mmHg and the corresponding arterial H^+ was 42 nmol/liter. Baseline PaO_2 was 108 mmHg; CPP averaged 126 mmHg. Increasing the $PaCO_2$ to 53 mmHg and the PaO_2 to over 400 mmHg in the normal cats: increased arterial H^+ to 66 nmol/liter, whereas MABP, ICP, and CPP remained essentially unchanged (Table 7).

After wounding with the animals breathing room air, the arterial blood gases were: pCO_2 30 mmHg and pO_2 102 mmHg. Arterial H^+ was 44 nmol/liter. The mean post wounding ICP was 62 mmHg but the CPP was 76 mmHg owing to a slight rise in MABP after wounding. With the post wounding hyperoxic-hypercapnic challenge the arterial pCO_2 rose to 54 mmHg and the pO_2 to 480 mmHg; H^+ increased to 70 nmol/liter. The ICP rose slightly to 67 mmHg but because the MABP fell from 138 mmHg to 130, the CPP decreased to 63 mmHg.

Table 6: Changes in rCBF in response to hypercapnic-hyperoxia (5% CO_2 + 95% O_2 breathing) before and after BMW. Pre-wound hypercapnic and normocapnic variables are compared; post-wound hypercapnic and normocapnic variables are compared. *Significant; $p<0.05$. Mean ± SE, (ml/100g/min)., (n=3)

	PRE-WOUND		POST-WOUND	
VARIABLES	NORMOCAPNIA	HYPERCAPNIA	NORMOCAPNIA	HYPERCAPNIA
PaCO2 (mmHg)	30.0	53.0	30.0	54.0
PaO2 (mmHg)	108.0	422.0	102.0	480.0
FRONTAL CORTEX	39.47 1.57	129.73* 13.92	30.77 6.02	34.77 10.38
PARIETAL CORTEX	40.20 2.82	76.37* 5.93	29.73 5.90	31.37 9.42
TEMPORAL CORTEX	22.93 1.22	73.57* 11.46	24.07 2.86	28.30 5.64
OCCIPITAL CORTEX	47.47 6.18	96.20* 8.85	31.57 7.30	32.63 9.07
PERIWOUND GRAY	34.53 1.46	94.47* 7.47	25.27 6.14	17.90 3.71
PERIWOUND WHITE	32.97 2.04	65.87* 1.90	27.00 6.08	19.43* 5.00
WHITE MATTER	29.67 1.77	63.07* 7.88	22.70 2.37	24.33 4.30
CAUDATE NUCLEUS	57.07 4.24	137.93* 7.87	46.03 13.05	60.50 16.43
HIPPOCAMPUS	22.93 2.24	57.07* 9.98	18.63 1.52	20.97 2.71
THALAMUS	42.80 3.22	107.00 18.19	33.20 4.06	39.43 7.58
RETICULAR FORMATION	40.30 3.68	87.43 16.75	29.97 4.22	33.10 7.00
BRAIN STEM & MEDULLA	26.90 1.65	64.90 14.55	21.90 1.18	25.70 2.85
CEREBELLUM GRAY	43.60 1.59	104.20* 19.43	30.43 3.57	38.93* 4.48
CEREBELLUM WHITE	43.03 3.97	73.47 12.91	31.30 2.00	37.30 0.93
TOTAL CBF	34.77 1.48	83.23* 9.37	26.93 3.67	29.80 5.34
SPINAL CORD	13.47 0.44	29.10* 5.98	15.27 1.05	21.80 4.65

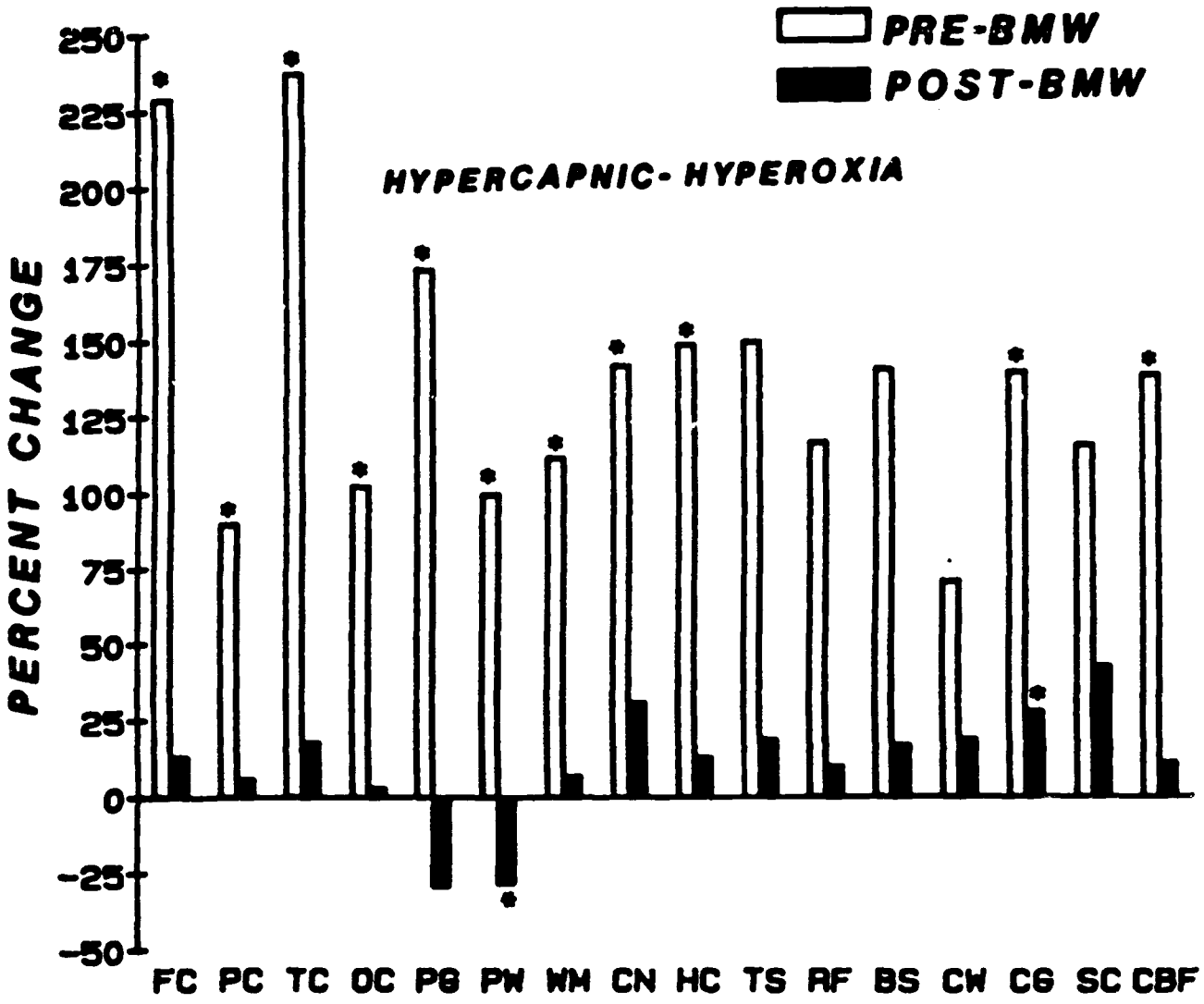


Figure 3: Percent change in rCBF following 10 min hypercapnic-hyperoxia (5% CO_2 + 95% O_2 breathing) before and after BMW. Before wounding, hypercapnic-hyperoxia produced extreme increases in the rCBF of the entire brain, whereas after BMW this vasodilatory effect was extremely abolished in all structures and was reversed to a vasoconstrictive effect in the periwound structures. Legends for brain structures, see Fig.1. *Significant; $p<0.05$.

Table 7: Changes in physiological parameters in response to hypercapnic-hyperoxia (5% CO_2 + 95% O_2 breathing) before and after BMW. Pre-wound hypercapnic and normocapnic variables are compared; post-wound hypercapnic and normocapnic variables are compared. *Significant; $p<0.05$. (Mean ± SE).

VARIABLES	PRE-WOUND		POST-WOUND	
	NORMOCAPNIA	HYPERCAPNIA	NORMOCAPNIA	HYPERCAPNIA
pH	7.38 0.02	7.18* 0.01	7.36 0.03	7.16* 0.02
[H+] (nmol/l)	42.17 1.53	66.37* 2.34	44.17 2.92	69.83* 2.89
PaO2 (mmHg)	108.00 9.07	422.00* 107.13	102.00 9.02	480.33* 54.39
PaCO2 (mmHg)	29.73 1.07	53.20* 1.96	29.53 0.52	53.67* 1.48
MABP (mmHg)	127.00 7.81	121.67 11.22	137.67 7.67	130.00 13.28
ICP (mmHg)	1.00 9.85	-0.66 15.21	61.67 15.81	67.33 14.10
CPP (mmHg)	126.00 6.56	122.33 14.15	76.00 16.37	62.67 21.61
CVR (CPP/CBF)	3.66 0.18	1.54* 0.29	2.78 0.29	1.98 0.49
HEART RATE/min	206.67 14.53	190.00 20.00	210.00 15.28	200.00 15.28
CARDIAC BLOOD FLOW (ml/100g/min)	152.33 9.13	157.67 8.11	191.67 27.83	177.67 33.62
HEMATOCRIT (%)	26.00 0.58	28.17 0.73	28.17 0.17	28.50 1.89

DISCUSSION

The purpose of these studies was to continue our investigation into the extent of the impairment in chemical regulation of the CBF caused by a missile wound to the brain. The chemical control of CBF was tested by exposing pentobarbital-anesthetized and ventilated cats to gas mixtures which produced either hypocapnia, hyperoxia, or both hyperoxia and hypercapnia.

1. Cerebrovascular response to acute hypocapnia induced by hyperventilation

The induction of hypocapnia by hyperventilation is recommended in the initial treatment of patients with acutely increased ICP (11,38,49) Clinical and experimental studies have shown that acute and prolonged hyperventilation may reduce trauma-induced increases in ICP (11,46). In theory, hyperventilation reduces $PaCO_2$ which increases brain extracellular fluid pH and results in vasoconstriction. Vasoconstriction reduces CBF, total intracranial blood volume (1,30,33,38,52,54,61) and ICP. Some studies have indicated that a reduction in ICP by hypocapnia may occur even in the presence of seriously impaired autoregulation (49).

In our brain wounding experiments under consideration here, prior to wounding ICP was 12 mmHg and total CBF averaged 31 ml/100g/min. Decreasing $PaCO_2$ from 31 mmHg down to 21 mmHg by hyperventilation did not reduce ICP even though CBF decreased to 26 ml/100g/min, a significant 18% reduction. The failure of this CBF reduction to decrease ICP may be explained if the intracranial blood volume didn't increase or if the cerebral spinal fluid production were increased or resorption were decreased. Even if intracranial blood volume were reduced, reference to the well known volume-pressure curve offers an explanation for the failure of hypocapnia to lower normal ICP (Figure 4).

When ICP is normal (to left of dotted line) even a large change in intracranial volume will not greatly affect ICP because fluids can freely exit the intracranial compartment via blood, CSF and other pathways. The 18% reduction in CBF (and presumed decrease in cerebral blood volume) did not provide a sufficient volume to effect an ICP change in our hypocapnic experiments.

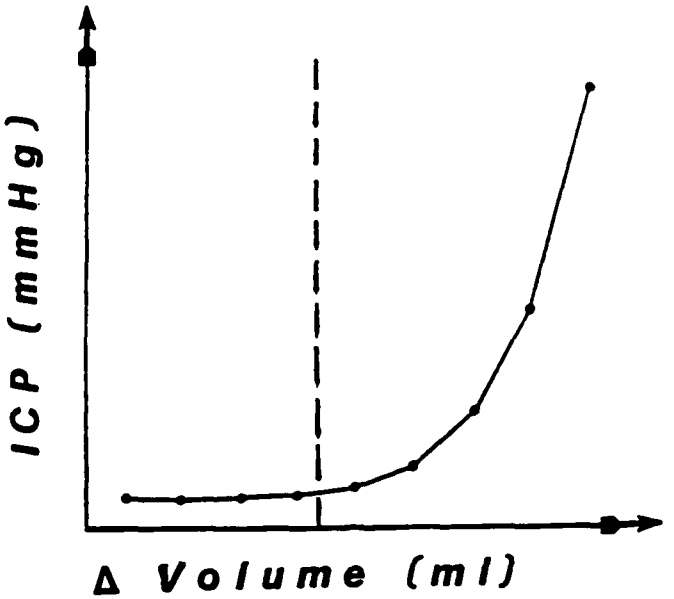


Figure 4: Intracranial pressure-volume relationship. A large change in intracranial volume (left of the dotted line) is needed before significant change in ICP will occur. Adapted from T.W. Langfitt: Increased Intracranial Pressure. Clin. Neurosurg. 16:436-471. 1969.

After wounding the ICP rose to 53mmHg and the normocapnic post wounding cerebral blood flow decreased to 23.1 ml/100g/min. Reducing $PaCO_2$ again to 21 mmHg achieved an 11% reduction in CBF but did not decrease ICP at all. Since an ICP elevation to 53 mmHg would undoubtedly displace the volume- pressure curve to the right of the dotted line where even a small change in intracranial volume would effect a large change in ICP, we infer that hypocapnia induced after brain wounding did not alter cerebral blood volume sufficiently in our model to cause a decrease in ICP.

While hyperventilation is widely advocated as a means of quickly controlling elevated ICP in humans, in our cat model it appears ineffective during a short period of hyperventilation following brain wounding. Reasons for this failure should be ascertained. Intracerebral blood volumes and their changes in response to $PaCO_2$ alterations both before and after brain wounding should be investigated. Clinically, data from brain injured children indicate that hyperventilation cannot control ICP levels of greater than 60mmHg (35). Possibly, the ICP of 53 mmHg observed in our cats after wounding approaches the level where hyperventilation becomes ineffective. The limits to the effectiveness of hyperventilation as an ICP-reducing therapy following brain wounding with lower and higher levels of ICP should, therefore, also be investigated so that following a brain wound in humans

one would know under what conditions one might apply hyperventilation with some hope of successfully reducing ICP. This knowledge would enhance the timely provision of optimal care following a brain wound. If hyperventilation is unlikely to be effective in reducing ICP, time should not be wasted using this therapeutic modality. Other means of reducing ICP should be immediately employed.

Table 8 indicates how rCBFs of selected brain regions were affected by BMW and how the rCBFs responded to hypocapnia both before and after wounding.

Table 8: Changes in rCBF in selected brain regions* and the response of rCBF to decreased $PaCO_2$ before and after brain wounding (from Table 1; rCBF in ml/100g/min)

$PaCO_2$ (mmHg)	Before Wounding			After Wounding		
	30	21		30	21	
	rCBF	rCBF	% Δ rCBF	rCBF	rCBF	% Δ rCBF
cerebral cortex	32.4	26.0	-20.0	23.4	21.0	-10.0
hippocampus	21.5	17.9	-16.7	17.5	16.0	8.6
cerebral white matter	26.1	21.3	-18.4	20.4	16.7	-18.1
caudate	43.8	37.8	-13.7	33.7	27.3	-19.0
thalamus	39.2	29.8	-24.0	27.5	23.2	-15.6
brain stem-medulla	29.9	25.7	-14.0	21.6	20.6	-4.6
cerebellum	42.0	33.7	-19.8	26.3	26.2	0.4
periwound	30.3	26.0	-14.2	27.2	20.2	-25.7

While all brain regions exhibited a relatively similar reduction in rCBF with hypocapnia prior to wounding, after brain wounding 4 different rCBF patterns to hypocapnia appeared: 1) the cerebral white matter and caudate showed about the same percent decrease in rCBF with decreased $PaCO_2$; 2) the cortex, hippocampus, thalamus and brainstem showed smaller rCBF decreases in response to decreased $PaCO_2$; 3) cerebellar blood flows showed no diminution of rCBF in response to hypocapnia indicating an almost total loss of vascular reactivity to this chemical stimulus following BMW; and 4) interestingly, the percentage decrease in blood

* Selected brain regions presented in Tables 8 to 11 were made from Tables 1,3, and 6. All cortical areas were combined; both cerebellar samples were combined as were both periwound rCBFs.

flow about the wound track increased with the hypocapnic stimulus after wounding.

Prior reports have indicated loss of chemical blood flow regulation in the brain following injury (80,81) but our data indicate that this loss is rather heterogeneous. Why blood vessels in the cerebellum should totally lose their reactivity to hypocapnia is unknown. Our prior work (Final Summary Report DAMD17-83-C-3145, 1987) has shown that immediately after wounding enormous increases in CSF prostaglandins occur. Cerebellar folia are highly bathed in CSF. Perhaps the observed loss of vascular reactivity observed in the cerebellum reflects CSF-mediated effects of prostaglandins or other substances released by wounding on cerebellar blood vessels.

In addition to totally abolishing cerebellar vascular reactivity, hypocapnia also greatly attenuated the brain stem-medulla vascular reactivity after brain wounding. Before wounding, hypocapnia caused a 14% decrease in the brain stem medulla rCBF; after wounding, the decrease was only ~5%. Both brain stem and cerebellum are supplied by the vertebral arteries. It would appear that the basivertebral complex is particularly susceptible to the loss of hypocapnic control of blood flow regulation following brain wounding. Since the brain wound in the right cerebral hemisphere did not involve arteries of the basivertebral complex directly, this physiological phenomenon would appear yet another indirect effect of brain wounding on brain structures far removed from the site of actual injury. It is interesting that the brainstem-medullary loss should be so great (a 65% loss of reactivity to hypocapnia) because many serious, post-wounding events occur in the brainstem.

Reasons why brain tissue about the wound track should show an increased responsiveness to the lowered $PaCO_2$ remain obscure. Our prior work on hypercapnia and hypoxia have also shown that, after wounding, blood vessels in the periwound tissues, do not react to chemical stimuli at all as does the vasculature in the rest of the cerebral cortex or white matter. Presumably, tissue factors from damaged brain diffuse into surrounding tissues and alter vascular reactivity. In this case they enhanced the vasoconstrictive effect of short term hypocapnia but by what mediators remains unknown.

A further problem to be delineated in our model is the effect of increased ICP alone on rCBF and how increased ICP alters the rCBF response to hypocapnia. The data just presented indicate that brain wounding tends to attenuate rCBF responsiveness to decreased $PaCO_2$ but in these experiments brain wounding also increased ICP to a mean of 53 mmHg. Possibly the alterations in rCBF following brain wounding were as a result of the ICP increase and breakdown of CBF autoregulation alone as opposed to the brain wound per se. However in normal cats ICP increase below 70 mmHg may not significantly affect CBF (21,22,27,31,62). This possibility requires investigation.

2. Cerebrovascular responsiveness to short term hyperoxia

Clinically, patients with brain injury, hypoventilation, and hypoxia are often given oxygen. In 1943 Dunke and Schmidt (19) measured carotid artery blood flow in Macaque monkeys by a flow meter and observed a decreased blood flow when 100% oxygen was inspired. Kety and Schmidt (33) quantitatively measured CBF (N_2O method) in awake men breathing 85% to 100% oxygen and found a significant total CBF reduction of 13%. Lambertsen et al (34) demonstrated a 15% decrease in CBF following 100% oxygen breathing in normal people using the N_2O method. Since the slight vasoconstrictive effect of normobaric hyperoxia (PaO_2 > 150 mmHg) on normal cerebral vessels is well known, we felt it important to assess the response of the wounded brain to hyperoxia. Brain wounding alone tends to reduce CBF somewhat if the ICP is elevated (41,59). We were concerned that the known effect of hyperoxia in reducing CBF might be significantly enhanced following a brain wound. If hyperoxia greatly decreased rCBF, its use might be contraindicated for resuscitative therapy following a BMW.

The rCBF responses to hyperoxia for selected brain structures both before and after wounding are summarized in Table 9.

Table 9: Changes in rCBF in specific brain regions and the response of rCBF to increased PaO_2 before and after brain wounding (From Table 3; rCBF in ml/100g/min)

PaO_2 (mmHg)	Before Wounding 129	280		After Wounding 128	280	
	rCBF	rCBF	% Δ rCBF	rCBF	rCBF	% Δ rCBF
cerebral cortex	41.5	38.5	-7.2	19.2	16.2	-15.4
hippocampus	17.6	17.2	-2.3	12.8	10.2	-20.3
cerebral white matter	30.8	28.6	-7.1	16.4	14.8	-9.8
caudate	35.5	31.8	-10.4	24.8	21.2	-14.5
thalamus	34.4	32.0	-7.0	21.4	20.2	-5.6
brain stem-medulla	24.4	34.0	+39.3	17.6	15.8	-10.2
cerebellum	36.6	50.9	+39.0	23.6	22.3	-5.5
periwound	36.6	31.6	-13.7	26.7	18.0	-32.6

Before brain wounding total CBF was 32.3 ± 6.1 ml/100g/min and following hyperoxia it was 33.8 ± 7.3 indicating no significant change.

Whereas hyperoxia caused a decrease in rCBF in many brain regions, blood flows increased in the brain stem-medulla and cerebellum. The dual vasoconstrictive and vasodilatory effect of arterial hyperoxia occurring simultaneously in different cerebrovascular beds has not been described before. The failure of hyperoxia to reduce total CBF as expected in our cats can be explained by sampling bias: relative to the size of the cerebral hemispheres few cerebral cortical samples were taken. Large flow increases occurred in the brain stem-medulla and cerebellum but these structures make up a smaller part of the brain. Brain stem-medulla and cerebellum were proportionately over represented in our sampling scheme. Prior to wounding all telencephalic structures exhibited rCBF reductions of the expected magnitude following hyperoxia.

Why the normal cat's brain exhibits this dual response to hyperoxia is unknown. Interestingly, flow increases to hyperoxia occurred in the vertebral artery distribution suggesting that this portion of the basivertebral circulation reacts differently to the hyperoxic stimulus than does the carotid system.

Brain wounding raised mean ICP to ~64 mmHg and, as expected, with this large ICP increase and with brain bood flow regulation disturbed, total CBF was reduced. Total CBF after wounding fell to 19.5 ml/100g/min. When the hyperoxic stimulus was given after wounding the ICP decreased to 60 mmHg (6%); total CBF still decreased significantly further to 17.0 ml/100g/min (-13%) indicating that the overall vascular response to hyperoxia remained intact after wounding. Following the BMW no rCBF increases to hyperoxia occurred in either the brain stem or cerebellum.

Following wounding, hyperoxia enhanced rCBF decreases in most brain structures. (eg cortex rCBF fell 7% with hyperoxia before wounding and 16% when hyperoxia was induced after BMW). This post wounding effect of hyperoxia on rCBF was particularly enhanced in periwound tissues where the hyperoxia-induced flow decrease more than doubled (from -14% before wounding to -33% afterwards).

Why the vasoconstrictive effect of hyperoxia following BMW should be augmented is unknown but the rCBF changes brought about in the brain stem and cerebellum are impressive: in response to hyperoxia before wounding cerebellar and brain stem rCBFs increased 39%; after wounding these rCBFs showed a 6% to 10% decrease to this stimulus.

If, after wounding rCBF is marginal in a particular brain structure in the normoxic state (eg hippocampus, Table 9) hyperoxia can decrease flow to that brain region dangerously. Our data also suggest that even though the vasoconstrictive effect of hyperoxia is enhanced in damaged brain about the wound track, in the usual case early after wounding periwound rCBF is not particularly depressed and flow reductions caused by hyperoxia would not be severe enough to cause tissue death in brain immediately adjacent to the missile track.

In these wounded cats, thirty minutes after the hyperoxic trial had been terminated all rCBFs increased to their pre-hyperoxic baselines (Table 4) except for rCBFs in the periwound tissues which remained decreased. This observation indicates that the vasodilatory mechanisms in brain not directly damaged by the missile remained intact but normal mechanisms causing vasodilation in physically damaged vessels are entirely lost. Why tissues directly adjacent to the wound track should enhance the vasoconstrictive effects of hyperoxia remain unclear but we hypothesize that this occurs because of local factors generated from direct tissue damage.

It has been hypothesized that the vasoconstrictive effect of hyperoxia on cerebral arteries may be from an enhanced sympathetic tonus modulated by endothelial prostacyclin synthesis (47) Prostacyclin, a potent vasodilator, modulates vascular adrenoreceptors. While the regional biosynthesis of prostacyclin in brain vasculature is not known, variability in its production in various parts of the brain, could explain the dual vasoconstrictive and vasodilatory effect of hyperoxia in normal brain and also the post wounding enhancement of rCBF flow decreases caused by hyperoxia. One might hypothesize that more prostacyclin is normally present in the basivertebral system and this vasodilator mediator is activated by the hyperoxic stimulus. After wounding, either the biosynthesis of prostacyclin is reduced throughout the brain or the sympathetic tonus is enhanced, creating only a pronounced vasoconstriction in both carotid and basivertebral vascular systems. Other scenarios impuning other mediators and receptor sites could also be invoked.

As stated in our previous discussion concerning hypocapnia and CBF, modulators released by wounding into the CSF bathing cerebral arteries might also be responsible for the post injury alterations in the effect of hyperoxia on CBF; or possibly brain stem centers controlling CBF may be affected consequent to the demonstrated "brain stem" effects of the brain missile wound.

Brain wounding appears to augment the vasoconstrictive effect of hyperoxia on CBF but this wound effect must be clearly differentiated from the effect of ICP alone. Control experiments where ICP is raised without wounding need to be done and the effect of hyperoxia examined.

Traditionally, hyperventilation-induced hypocapnia has been used to reduce ICP (see prior section). Although nothing is known on the effect of prolonged normobaric hyperoxia on rCBF or ICP it would be wise to investigate whether hyperoxia would be more effective than hypocapnia in reducing elevated ICP when brain blood flow autoregulation is disturbed.

3. Cerebrovascular responsiveness to short term hyperoxia coupled with hypercarbia

Given individually, oxygen and carbon dioxide have opposite effects on CBF. Increasing PaO_2 tends to reduce CBF (see prior section) while increasing $PaCO_2$ tends to increase it. While the effect of hypercarbia alone in increasing CBF is abolished after wounding (see Annual Report DAMD17-86-C-6098, April, 1989), the vasoconstrictive effect of hyperoxia alone after wounding persists and its vasoconstrictive effect is even increased; (see prior section, Table 9). Table 10 summarizes the effect of elevated PaO_2 and $PaCO_2$ given simultaneously on rCBF.

Table 10: Changes in rCBF (ml/100 mg/min) in specific brain regions in response to increased PaO_2 and $PaCO_2$ before and after wounding

	Before Wounding			After Wounding		
PaO_2-$PaCO_2$ mmHg	108-30	442-53		102-30	480-54	
	rCBF	rCBF	% Δ rCBF	rCBF	rCBF	% Δ rCBF
cerebral cortex	37.5	94.0	+160.5	29.0	31.8	+9.8
hippocampus	22.9	57.1	+149.3	18.6	21.0	+12.9
cerebral white matter	29.7	63.1	+112.5	22.7	24.3	+7.0
caudate	57.1	137.9	+141.5	46.0	60.5	+31.5
thalamus	42.8	107.0	+150.0	33.2	39.4	+18.7
brain stem medulla	26.9	64.9	+141.3	21.9	25.7	+17.4
cerebellum	43.3	88.8	+105.2	30.8	38.1	+23.7
periwound	33.8	80.2	+137.3	26.2	18.6	-29.0

Before brain wounding when the normal brain was exposed to increased PaO_2 and increased $PaCO_2$ simultaneously the vasodilatory effect of the elevated $PaCO_2$ overrode the vasoconstricting effect of the elevated PaO_2. Total CBF rose significantly from 34.8 to 83.2 ml/100g/min (+139%) (See Table 9 for effect of increased PaO_2 alone). All rCBFs increased significantly as well. Theoretically, this would provide maximal brain tissue oxygenation under normobaric conditions.

After brain wounding the ICP rose to 62 mmHg and total CBF was reduced from ~35 ml/100g/min before wounding to ~27 ml/100g/min afterwards. When, after wounding, the $PaCO_2$ was increased along with the PaO_2 flow reductions consequent to hyperoxia were prevented in all brain areas save that of the periwound tissue. This blood flow response was in great contrast to the rCBFs following BMW and hyperoxia alone. When

hyperoxia alone was introduced after wounding this stimulus tended to enhance rCBF reductions (Table 9).

Table 10 indicates that following brain wounding the ability for an elevated $PaCO_2$ to increase CBF generally throughout the brain was attenuated or even completely abolished in the white matter of the wounded hemisphere or in brain tissue adjacent to the wound track. Furthermore, after wounding in the periwound area, the ability of increased $PaCO_2$ to ameliorate the vasoconstrictive effect of hyperoxia was completely lost. When PaO_2 was increased to > 280 mmHg and $PaCO_2$ was 33 mmHg periwound rCBF fell 33% (Table 9); when PaO_2 was 480 mmHg and $PaCO_2$ was increased to 54 mmHg periwound rCBF fell 29% (Table 10). Comparison of the percent difference in rCBF after wounding during hyperoxia alone (last column in Table 9) with % difference in rCBF after wounding during hyperoxia-hypercapnia (last column in Table 10) shows:

1) Even though the vasodilatory effect of increased $PaCO_2$ is greatly attenuated after wounding it is able to overcome the vasoconstrictive effects of increased PaO_2 which appear to be enhanced after wounding.

2) In damaged brain about the wound track the vasoconstrictive effect of the hyperoxia predominates. Any tendancy for elevated $PaCO_2$ to increase rCBF in damaged brain about the wound track is lost.

THESE EXPERIMENTS SUGGEST THAT AFTER BRAIN WOUNDING rCBF IS MAINTAINED BETTER BY SIMULTANEOUSLY INCREASING THE $PaCO_2$ WHEN INCREASING THE PaO_2. UNFORTUNATELY THIS INSPIRED GAS COMBINATION DOES NOT APPEAR TO IMPROVE rCBF IN DAMAGED BRAIN DIRECTLY ABOUT THE MISSILE TRACK.

General significance of these experiments on chemical regulation of rCBF following missile wounding of the brain:

1) Tables 8-12 indicate that chemical cerebral blood flow regulation is greatly perturbed following brain wounding. This disturbance is widespread throughout the brain and is particularly severe in directly damaged brain about the missile track. These disturbances in chemical rCBF regulation are quite heterogeneous. These changes in chemical regulation of CBF are schematically represented in Table 11.

Table 11: Schematic Representation Demonstrating Normal and Post Wound (W) rCBF Responses in Various Brain Areas

Brain Area	rCBF response to Hypocarbia		rCBF response to Hyperoxia		rCBF response to Hypercarbia + Hyperoxia	
	Pre W	Post W	Pre W	Post W	Pre W	Post W
Cortex						
White Matter						
Brainstem-Medulla						
Cerebellum						
Periwound						

2) Because of this disturbance in chemical blood flow regulation, following brain wounding measures as hyperventilation-induced hypocapnia may prove ineffectual in reducing CBF, cerebral blood volume and ICP. After wounding rCBF appears to be better maintained by increasing $PaCO_2$ and PaO_2 simultaneously than by increasing PaO_2 alone. Additional experiments should be done to reconfirm and substantiate these findings because these findings have direct clinical applicability for the treatment of those with brain injury.

3) Normally, a coupling exists between cerebral blood flow and cerebral metabolism. Such a coupling is requisite for normal brain function. Disturbances in rCBF autoregulation consequent to brain wounding theoretically would have the potential for uncoupling brain blood flow from brain metabolism and thereby causing additional brain damage. The potential for this would appear greatest about the wound track where rCBF tends to decrease with hypocapnia, hyperoxia or the combination of hyperoxia plus hypercapnia. We know of no experimental data which examines the regional coupling or uncoupling between blood flow and metabolism following brain wounding and how this may be affected by the lack of autoregulation. Knowledge of this for brain about the wound track would be particularly important if damaged, marginally viable brain near the missile wound track is to be salvaged.

Section B: The Effect of Wounding On Selected Physiologic Variables In Spontaneously Breathing, Non-Respirated Cats

PAGE PURPOSELY BLANK

SECTION B: THE EFFCT OF WOUNDING ON SELECTED PHYSIOLOGIC VARIABLES IN SPONTANEOUSLY BREATHING, NON-RESPIRATED CATS

INTRODUCTION

Many of our prior experiments, especially those on CBF autoregulation, were performed on anesthetized, ventilated cats whose respiratory variables were kept normal after wounding by respiratory adjustments. In order to circumvent this artificial state in this study we evaluated many physiological variables both before and after brain wounding in spontaneously breathing animals.

We wished to answer the following questions:

1. Could death following missile wounding consistantly be ascribed to any specific physiological variable? Particularly crucial variables included: intracranial pressure (ICP), cerebral perfusion pressure (CPP), brain stem blood flow (BSBF), and cardiac output (CO), and ventilaton (V)

2. Could any clinically accessible physiological variables indicate which brain wounded animals would die after missile injury? Such variables included: mean arterial blood pressure (MABP), ICP, and CPP; EEG; heart rate (HR), and EKG; respiratory frequency (f) tidal volume (V_T), and ventilation (V).

METHODS

Two groups were used in this study: unwounded controls (Group IV, N=4) and cats receiving a brain wound (Group V; N=15). All cats were anesthetized and surgically prepared as described under General Methods (p 5) but these animals were not paralyzed and breathed spontaneously through the entire experiment. Following cannula placement the animals were put into the stereotaxic frame and prepared as indicated in the General Methods section.

For unwounded controls (Group IV), a microsphere injection for blood flow and cardiac output (CO) determinations was made about 10 minutes before "zero time" (corresponding to the time of wounding in injured cats) and then 5,20,45 and 90 minutes afterward. Arterial blood samples for blood gas analysis and hematocrit were taken immediately after each blood flow measurement.

For animals receiving a BMW (Group V), a microsphere injection was made about 10 minutes prior to wounding to determine control rCBF and CO. The cats were then wounded at "zero time". We intended to inject microspheres at 5,20,45,90 minutes after wounding in all cats but 3 cats died within a few minutes of wounding and 8 died from 8 to 41 minutes after being wounded. These had only one or two microsphere injections. Four cats completed the entire experiment. As for unwounded cats arterial blood samples for blood gas analysis and hematocrit concentration were taken immediately after each microsphere injection.

In contrast to the intermittent blood flow, CO, blood gas and pH determinations, ICP, MABP, CPP, EEG, HR, EKG, f, tidal volume (V_T) and ventilation (V), ($V = f \times V_T$) were continually monitored in both groups IV and V throughout the entire experiment.

Experimental Procedures For Determination of CO:

CO was calcuated by the formula:

$$CO = \frac{\text{Reference Flow X Total Injectate}}{\text{Reference Count}}$$

The reference blood was withdrawn at a rate of 1 ml/min for 90 sec and the reference count (RC) was determined by a gamma counter. The total injectate (TI) of each sample was determined using the following procedures:

1) The original and dry microspheres were first dissolved in 9.9 ml saline plus 0.1 ml Tween 80.

2) Microspheres in the original solution were then vigorously shaken and a 100-150 mg sample (sample weight:SW) was removed.

3) The microspheres in the sample were then diluted and divided equally into 10 counting vials. The radioactivity of these vials was determined along with tissues and blood samples from each experiment. The total counts in these 10 vials is equal to sample count (SC).

4) Before each experiment, the exact weight of 1 ml disposable plastic syringe with needle (#26), containing a specific radioisotope was determined. The weight of the syringe was again determined after injection of the microspheres. Thus, the weight of the injectate (IW) could be calculated.

5) Since SW, SC and IW are known, the total injectate count (TI) can be calculated as:

$$TI = \frac{SC \times IW}{SW}$$

In separate experiments residual counts in the syringe and needle were measured in 8 samples for our 5 microspheres (40 measurements). Residual counts ranged from 0.5% to 4.5% of the injectate. As this slight residual was ignored in our experiments, our calculated COs may be slightly overestimated. Our control CO value in 16 cats was (142 $\pm$ 22 ml/kg/min) in close agreement with reported CO values of pentobarbital-anesthetized cats (151 $\pm$ 18 ml/kg/min) (60). One of our 16 cats, however, had a control CO of 440 ml/Kg/min, quite high. Two subsequent CO determinations were not made in this cat and data from this animal could not be used for statistical analysis. Excluding this animal mean control CO was 122 $\pm$ 9 ml/Kg/min.

Statistical Analysis: All data are expressed as mean $\pm$ SE. Statistical evaluation of the data in Groups IV and V was made first by analysis of variance followed by Tukey's test. Comparison of physiological data between surviving and non-surviving cats was made by an unpaired t-test. Significance was determined by a $p < 0.05$.

RESULTS

Unwounded Control Cats N=4. The mean systemic and intracranial pressures plus respiratory variables from 10 minutes before "zero time" and then at 5 min, 20 min, 45 min, and 90 min after "zero time" are presented in Table 12. Cardiac outputs tended to decrease over 90 minutes of the experiment but the large SEs of the mean COs precluded the 90 minutes CO decrease from being significant. No significant changes in any cardiovascular or respiratory variable occurred during the entire experiment. The rCBFs also showed no significant changes at the different time points, Table 13.

Wounded Cats (N=15). Fifteen spontaneously breathing cats were wounded. The three cats which died within a few minutes of wounding quickly became apneic and developed flat EEGs indicating brain death, presumably from increased ICP and generalized cerebral ischemia. No postwounding blood flow measurements were made in these 3 cats and we report no data from them.

Physiological variables in the 4 surviving and 8 non-surviving brain wounded cats

MABP ICP and CPP: After wounding MABP both in survivors and non-survivors rose to almost twice control then fell gradually towards baseline. In the 4 survivors mean MABP remained steady and near control for the remainder of the experiment but in the 8 non-survivors, it was quite variable after wounding (Figures 5 and 6). Mean ICP in both survivors and non-survivors was acutely increased to > 60mmHg consequent to wounding but then the ICP elevations gradually fell to a static increase of 30 to 40 mmHg in both groups Five and 20 minutes after wounding the mean ICP in survivors was 47±13 mmHg and 30±4 mmHg while in non-survivors it was 45±11 mmHg and 31±16 mmHg; CPP in survivors was 45±9 mmHg and 58±9 mmHg while in non-survivors it was 54±14 mmHg and 63±6 mmHg. The static increase in mean ICP were steady in survivors but quite erratic in non-survivors. Physiological data from survivors and non-survivors are presented in Tables 14 and 15.

Changes in heart rate : In the 4 survivors, after a transient bardycardia, mean heart rate (HR) returned to normal within 5 to 10 min after wounding. After wounding, the 8 non-survivors were clearly unable to stabilize their HRs. The mean HR in this group remained depressed and fluctuated until death (Figure 7).

Changes in Respiratory Variables: Following brain wounding both survivors and non-survivors initially developed a significant reduction in mean respiratory frequency (f), (Figure 8). The 4 survivors regained their prewound mean respiratory rate quickly while the 8 non-survivors failed to regain a normal mean f. Respiratory frequency in this group continued to fall until death. Survivors maintained normal ventilation, V while non-survivors exhibited 49 to 58% decrease in V (Table 15).

Table 12: Changes in the physiological parameters of unwounded spontaneously breathing cats during 5 consecutive measurements, showing no significant differences over a 100 min period, (Mean ± SE),(n=4; CO n=3).

		POST-WOUND			
VARIABLES	CONTROL	5 min	20 min	45 min	90 min
pH	7.37 0.05	7.38 0.02	7.39 0.03	7.36 0.04	7.30 0.04
[H+] (nmol/lit)	43.00 4.62	41.33 2.03	40.67 3.18	43.67 4.10	50.00 4.93
PaCO2 (mmHg)	31.97 3.44	30.70 3.48	30.33 3.43	30.10 4.00	33.20 3.69
PaO2 (mmHg)	108.10 7.58	110.23 7.36	116.77 8.86	117.87 6.48	113.20 5.28
MABP (mmHg)	98.90 6.97	96.10 8.46	92.33 14.36	96.53 11.75	104.43 9.87
ICP (mmHg)	9.67 1.33	12.33 2.19	19.67 7.31	22.00 9.17	21.83 10.37
CPP (mmHg)	89.23 5.64	84.10 7.74	72.67 10.33	74.53 6.43	82.60 3.86
CVR (CPP/CBF)	2.44 0.33	2.18 0.38	1.96 0.46	2.75 0.69	2.58 0.28
HEART RATE/min	188.00 21.17	192.00 18.33	188.00 14.42	200.00 10.58	208.00 17.44
HEMATOCRIT (%)	27.33 3.76	28.33 2.96	27.00 3.79	27.00 3.79	25.67 3.28
f/min	26.67 0.88	28.00 0.00	33.67 0.67	31.67 4.26	34.00 6.08
Vt (ml)	49.67 4.37	45.93 7.79	41.33 5.70	41.67 4.67	37.00 4.16
V (lit/min)	1.34 0.18	1.34 0.12	1.31 0.18	1.30 0.04	1.28 0.10
CO (ml/kg/min)	104.00 5.00	98.00 8.00	101.00 15.00	91.00 11.00	70.00 26.00

Table 13: Changes in rCBF in unwounded spontaneously breathing cats during 5 consecutive measurements showing no significant differences over a 100 min period. Mean ± SE, (ml/100g/min).

		POST-WOUND			
VARIABLES	CONTROL	5 min	20 min	45 min	90 min
FRONTAL CORTEX	42.33 4.33	50.00 6.03	47.50 6.25	40.10 3.69	49.80 2.89
PARIETAL CORTEX	38.00 4.16	44.00 3.00	40.33 1.20	35.33 2.85	44.47 3.51
TEMPORAL CORTEX	27.80 2.04	27.73 2.97	26.43 1.27	23.67 0.67	28.13 3.00
OCCIPITAL CORTEX	41.67 4.91	55.23 9.41	49.00 1.00	39.00 3.79	48.00 3.51
WHITE MATTER	35.20 2.62	33.47 2.44	33.47 2.94	30.33 2.73	35.00 2.52
CAUDATE NUCLEUS	60.03 2.62	59.67 6.33	70.87 8.60	56.33 2.03	66.33 10.48
HIPPOCAMPUS	28.83 3.61	28.83 3.47	28.33 5.46	27.20 4.45	26.33 3.38
THALAMUS	56.77 14.67	55.50 5.48	61.90 9.47	51.33 6.98	62.67 7.88
HYPOTHALAMUS	25.60 4.21	22.50 1.76	25.53 1.44	21.13 2.52	29.20 3.75
RETICULAR FORMATION	51.63 8.23	54.17 6.50	66.27 2.32	47.50 3.55	53.33 4.10
BRAIN STEM & MEDULLA	30.27 8.38	28.57 4.49	37.67 3.76	25.00 2.08	26.67 2.60
CEREBELLUM GRAY	54.90 12.37	54.67 7.36	67.33 10.27	38.00 3.51	36.50 4.54
CEREBELLUM WHITE	57.40 16.02	51.67 9.39	50.53 5.80	33.87 1.27	31.67 1.20
TOTAL CBF	39.30 5.95	41.03 1.32	43.87 1.78	32.93 1.94	37.23 2.17

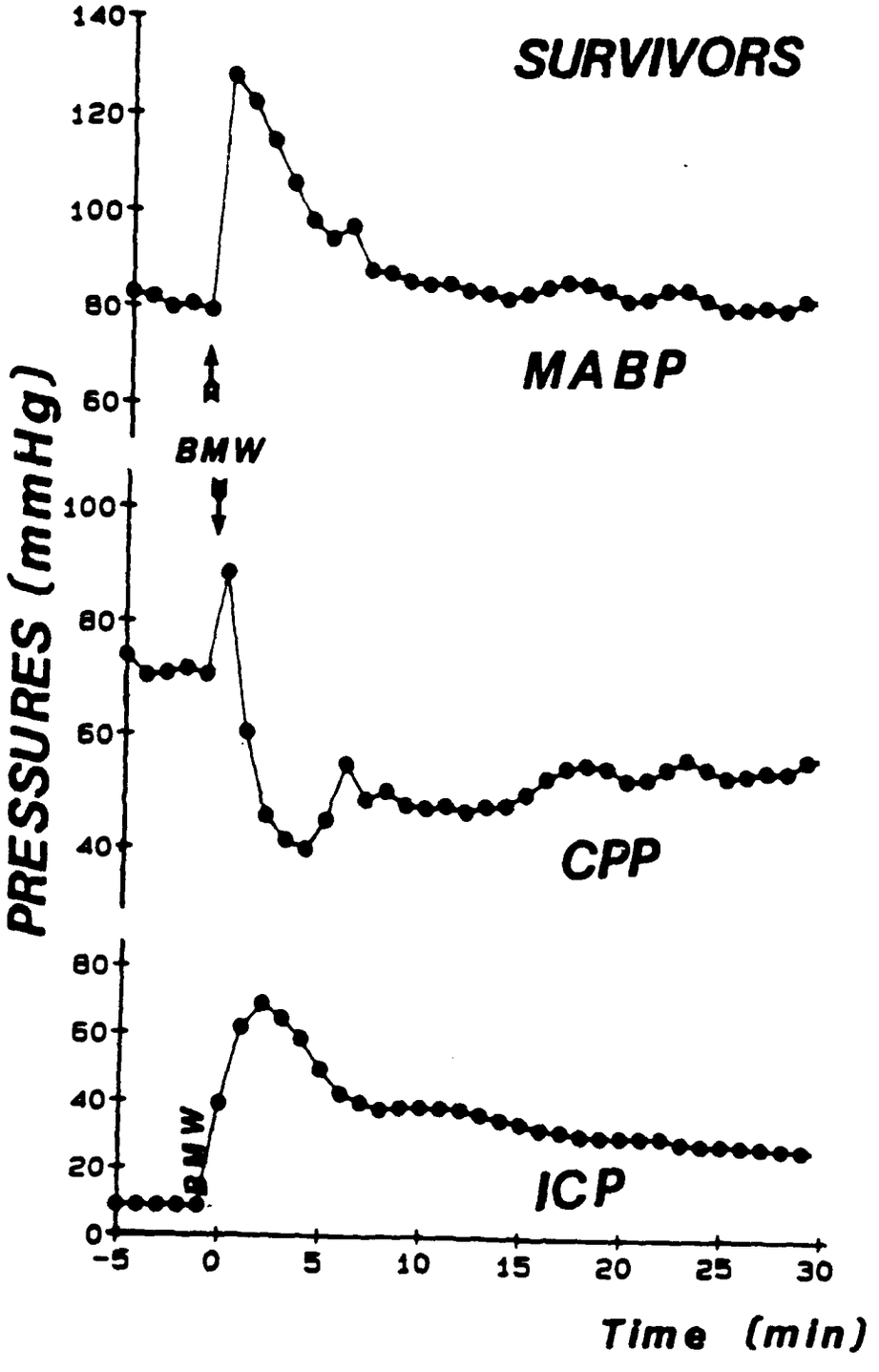


Figure 5: Changes in systemic and intracranial pressures in 4 surviving cats following BMW. The initial increases in MABP and ICP and reduction in CPP reached steady-state levels within 5 to 10 min after wounding. The MABP stabilized at normotensive levels while a reduced CPP and an increased ICP, at levels which do not effect CBF, were maintained.

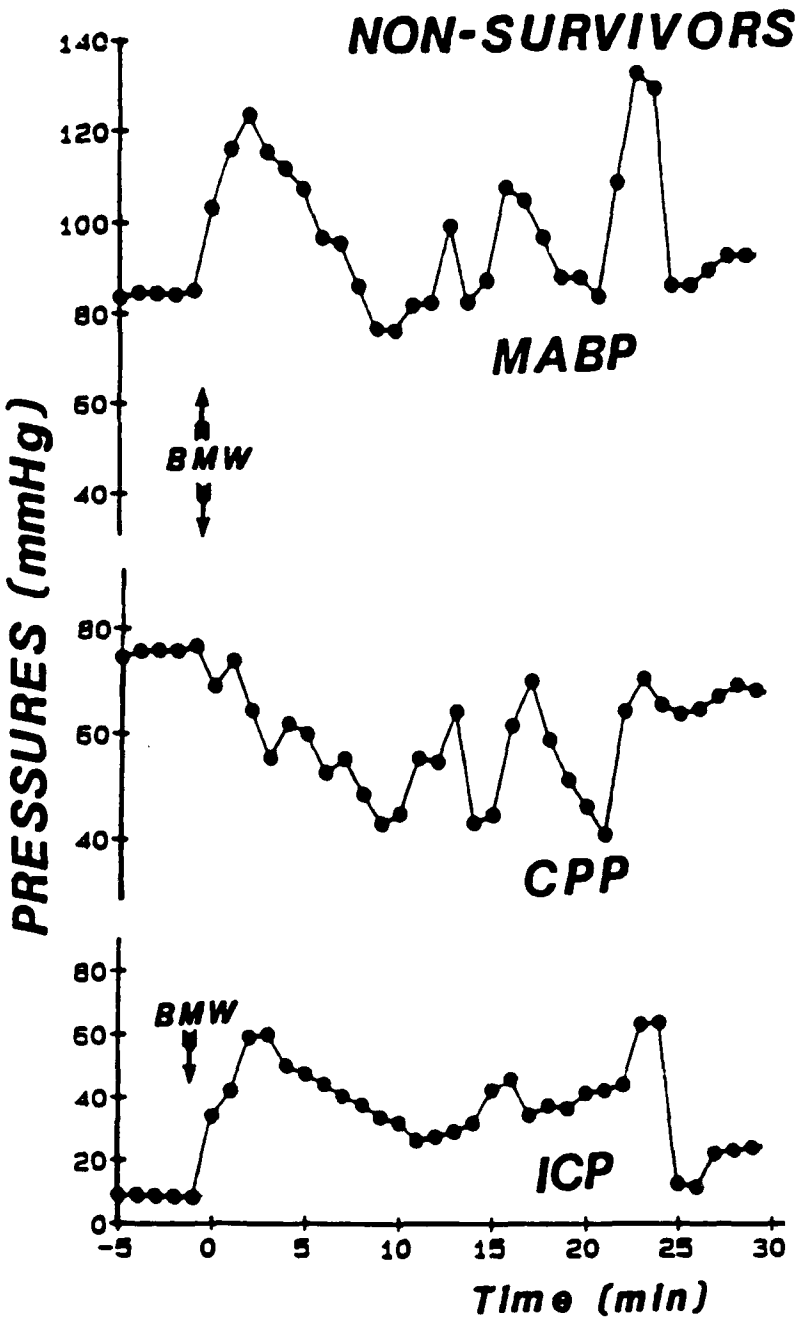


Figure 6: Changes in systemic and intracranial pressures in non-surviving cats following BMW. Starting 8 min after BMW the number of cats was gradually reduced because of death. Number of cats were 8, 3 and 2 at 5, 25 and 45 min post-wounding respectively. Unlike survivors (Fig.9) the non-survivros developed unstable changes in MABP, CPP and ICP after wounding indicating a brain stem effect of the BMW. Despite these fluctuations, the MABP was maintained at a normotensive level. The highest levels of ICP in the non-surviving cats were also no greater than those observed in survivors, and the CPP levels were adequate even at the lowest levels of fluctuation.

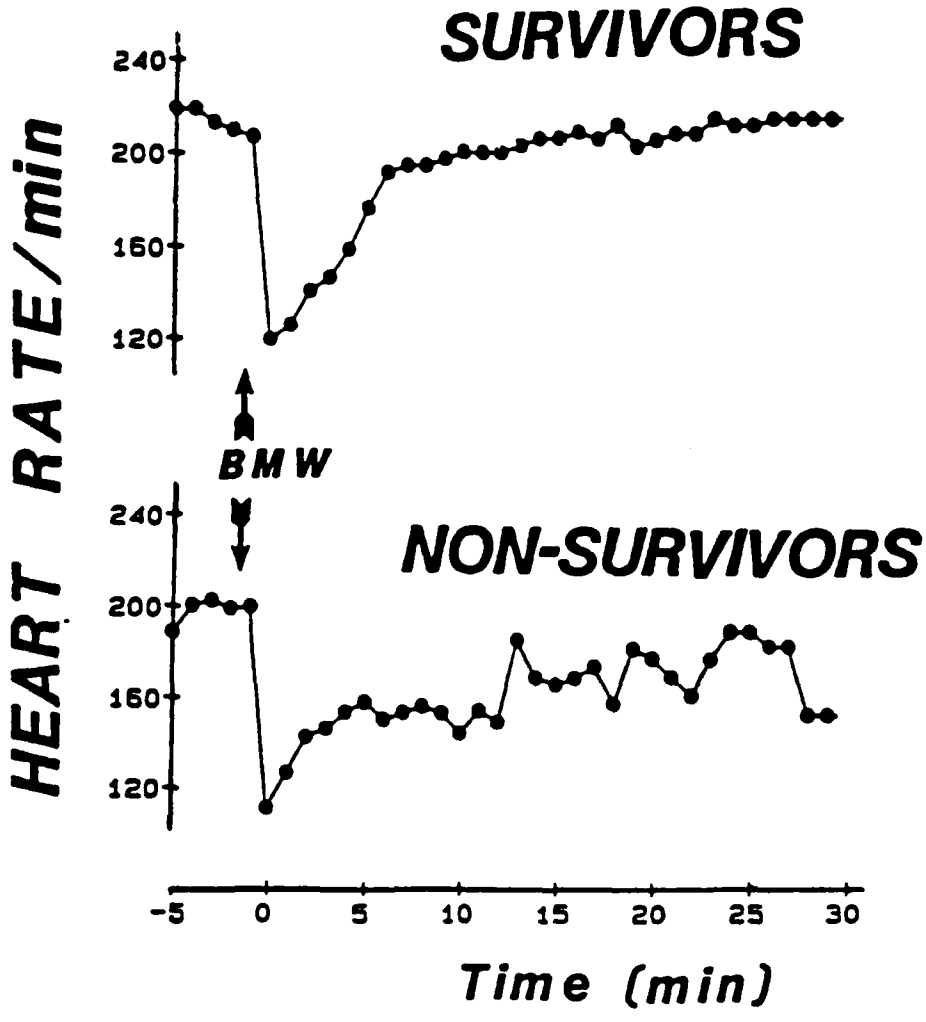


Figure 7: Comparison between changes in the heart rate in surviving (n=4) and non-surviving (n=8-3) cats following BMW. After initial arrythmias and bradycardia immediately after wounding the cardiac rhythm in survivors is quickly stabilized, while in non-survivors it fluctuates, indicating damage to cardiovascular centers in the brain stem.

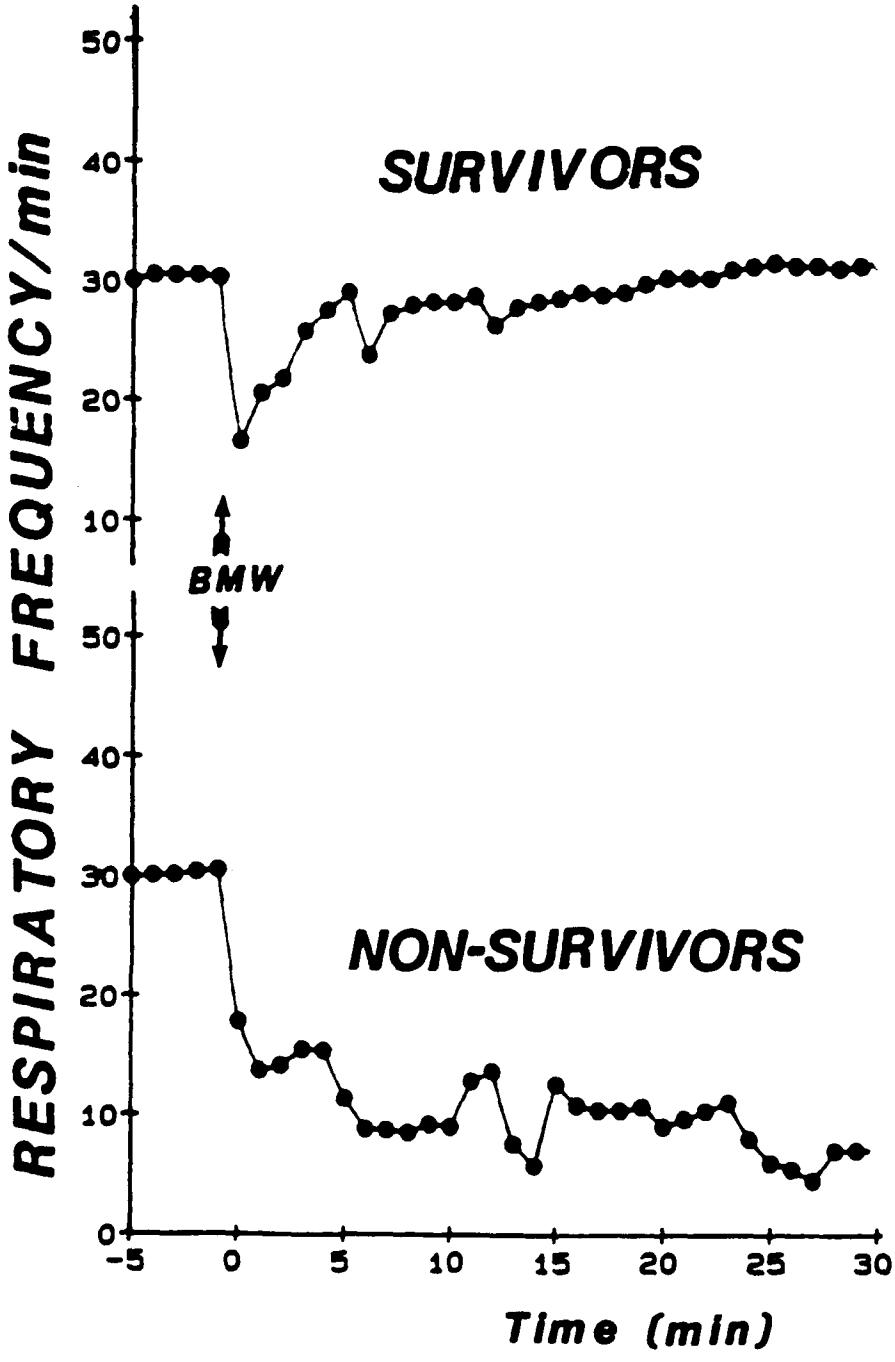


Figure 8: Comparison between changes in respiratory frequency (f) in surviving (n=4) and non-surviving (n=8-3) cats following BMW. The inital reduction in f after wounding was reversed in survivors within 5 min. The non-survivors, on the other hand, are unable to restore a normal f, indicating possible damage to the brain stem and medullary control centers of breathing.

Table 14: Changes in physiological parameters before (control) and at 5, 20, 45 and 90 min after BMW in 4 surviving cats. *Significant; $p<0.05$; control data are compared to post-wounding measurements, (Mean ± SE).

VARIABLES	CONTROL	5 min	20 min	45 min	90 min
pH	7.37 0.02	7.31 0.03	7.35 0.01	7.36 0.01	7.35 0.02
[H+] (nmol/lit)	41.75 2.02	48.50 2.90	44.00 1.29	42.75 1.31	44.00 1.83
PaCO2 (mmHg)	31.40 2.55	34.15 3.20	32.05 2.13	30.12 1.67	30.07 1.77
PaO2 (mmHg)	114.45 3.23	114.70 5.05	115.80 3.59	116.62 2.30	113.35 3.48
MABP (mmHg)	87.08 8.11	92.25 5.24	87.15 5.11	83.17 4.83	88.75 6.40
ICP (mmHg)	9.25 0.85	47.37* 12.63	30.00* 4.49	25.88* 4.50	25.13* 4.60
CPP (mmHg)	77.82 8.51	44.87* 8.89	57.65 9.00	57.35 9.22	63.67 10.08
CVR (CPP/CBF)	1.96 0.16	1.71 0.33	1.83 0.32	2.08 0.28	2.63 0.19
HEART RATE/min	222.00 15.87	174.00 24.74	207.00 15.78	204.00 17.66	201.00 21.56
HEMATOCRIT (%)	26.38 1.25	26.63 2.30	23.88 1.48	23.25 1.61	23.88 1.64
f/min	29.25 5.28	26.00 4.60	30.25 6.52	34.25 6.01	33.00 7.94
Vt (ml)	55.33 11.28	46.97 6.94	57.75 6.63	52.62 3.70	53.90 10.08
V (lit/min)	1.64 0.22	1.55 0.26	1.62 0.27	1.72 0.22	1.66 0.22
CO (ml/kg/min)	150.00 22.00	167.00 14.00	167.00 27.00	148.00 22.00	134.00 5.00

Table 15: Changes in physiological parameters before (control) and at 5 min (n=8) and 20 min (n=3) after BMW in non-surviving cats. *Significant: $p<0.05$; control data are compared to post-wounding measurements. Mean ± SE, (ml/100g/min).

VARIABLES	CONTROL	5 min*	20 min	45 min	90 min
pH	7.37 0.01	7.22* 0.04	7.25 0.05		
[H+] (nmol/lit)	42.87 1.16	62.00* 5.61	56.00 6.00		
PaCO2 (mmHg)	31.86 1.06	48.92 4.40	47.55 4.75		
PaO2 (mmHg)	110.60 1.94	69.12* 8.57	74.15* 1.95		
MABP (mmHg)	81.02 6.41	99.07 8.64	93.70 13.40		
ICP (mmHg)	6.25 1.52	44.71* 10.91	30.67 15.50		
CPP (mmHg)	74.77 6.76	54.21 14.39	63.03 6.09		
CVR (CPP/CBF)	2.21 0.43	3.08 1.25	2.29 0.64		
HEART RATE/min	199.50 14.51	159.00 16.00	176.00 21.17		
HEMATOCRIT (%)	25.38 1.53	27.63 1.55	28.50 4.50		
f/min	29.50 4.20	17.50 4.87	13.33* 5.21		
Vt (ml)	52.01 7.10	56.30 11.73	91.47 31.27		
V (lit/min)	1.47 0.19	0.62 0.23	0.75 0.28		
CO (ml/kg/min)	115.00 12.00	83.00 18.00	126.00 15.00		

Changes in mean CBF Post wounding blood flows in the 4 survivors were mostly unchanged from 5 to 45 minutes after wounding (Table 16) but by 90 minutes total CBF had fallen significantly from a control level of 42 ml/100g/min to 31 ml/100g/min. Mean rCBF was significantly reduced in the occipital cortex at 45 min post-wounding whereas parietal and frontal cortices, showed significant flow reduction only 90 min after wounding. A temporary and significant hyperperfusion (46% to 84% increase) occurred in periwound brain 5 minutes after injury (see Annual Report DAMD17-86-C-6098; September, 1987) but by 90 minutes after BMW these periwound blood flow increases had disappeared and periwound hypoperfusuion was evident; blood flows about the wound track were only 53% to 77% of their control values. The flow reduction in the periwound gray, from 44.3 to 23.3 ml/100g/min (-47%), was among the most severe rCBF reductions noted after wounding yet this rCBF decrease did not reach an ischemic level. Whether periwound blood flow would be reduced further at a later time and this tissue then be at risk for ischemia-reperfusion injury should be tested in future experiments. Despite these significant post wounding rCBF decreases (about the wound track plus occipital, frontal and parietal cortices) regional ischemia did not occur in other brain structures. Thus, the CBF patterns in surviving, spontaneously breathing cats resembled that of wounded, normotensive cats which were artifically ventilated (See Annual Report Contract DAMD17-86-C-6098, April, 1989).

Cerebral blood flow in non-survivors was fundamentally different from survivors, Table 17. Instead of maintaining a relatively constant total CBF for up to 20 minutes after wounding as did survivors, mean total CBF in non-survivors 5 minutes after wounding decreased from 43 ml/100g/min to 34 ml/100g/min (-19%). Regional cerebral blood flows followed a similar pattern: eg brain stem-medullary blood flows fell from 33 ml/100g/min to 26 ml/100g/min (-23%). No periwound hyperperfusion occurred in this group. None of these total rCBF decreases, however, were statistically significant. Perusal of Figure 9, however, reveals that the precent change in rCBF from control levels immediately after wounding was fundamentally different in non-survivors and survivors: virtually all % rCBF changes in non-survivors showed a decrease (unpaired t-test), (Fig 9). Since the mean ICP and CPP in survivors and non survivors was very nearly equal CBF differences between survivors and non survivors cannot be ascribed to ICP or CPP differences.

Cardiac Output Survivors maintained a stable CO for the entire 90 minute post wounding period. Mean COs ranged from 134±5 ml/kg/min to 167±14 ml/kg/min. Non-surviving cats had a mean control CO of 115±12 ml/kg/min. Five minutes after wounding mean CO fell to 83±18 ml/kg/min but this 28% reduction was not significant. Twenty minutes after wounding CO in the 3 remaining cats of this non-surviving group had rebounded to 126±15 ml/kg/min.

EKG and EEG: Besides bradycardia, severe cardiac arrythmias and EKG abnormalities occurred in both surviving and non-surviving cats starting 2-3 sec after wounding. EKG abnormalities included large increases in the amplitude of the Q-R-S complex, T-wave peaking and escape beats. These abnormalities usually disappeared within 5 to 10 min after wounding in survivors and non-survivors.

One cat which unlike all other non-survivors had only a very short transient apnea of 5 sec immediately after wounding, reestablished a normal sinus rhythm after wounding but died from sudden cardiac fibrillation 26 min post wounding. In this cat about 1 min before cardiac failure, a sudden increase in ICP from a post-wounding peak of 68 to 105 mmHg and MABP from a peak of 120 to 175 mmHg occurred. MABP suddenly fell to near zero mmHg at the end of cardiac fibrillation and before a permanent apnea ensued. Thus, cardiac arrest clearly preceded both apnea and EEG flattening (Figure 10).

In both survivors and non-survivors EEG amplitude and frequency were reduced. In 2/4 survivors, however, a slight improvement was noticeable 90 min after wounding. While in non-survivors the EEG amplitude continued to be depressed until it became flat at the onset of the permanent apnea.

Table 16: Changes in rCBF before (control) and at 5, 20, 45 and 90 min after BMW in 4 surviving cats. *Significant; $p<0.05$; control data compared to post-wounding measurements. Mean ± SE, (ml/100g/min).

VARIABLES	CONTROL	5 min*	20 min	45 min	90 min
FRONTAL CORTEX	49.50 6.36	56.75 8.19	51.29 11.13	44.00 6.28	38.00* 1.53
PARIETAL CORTEX	47.00 7.67	45.25 6.87	48.25 13.68	40.25 8.75	33.33* 2.60
TEMPORAL CORTEX	33.50 4.48	34.50 5.19	32.50 7.17	31.75 5.27	27.33 1.33
OCCIPITAL CORTEX	54.25 7.59	54.25 7.09	53.75 14.87	41.00* 5.82	34.00* 2.89
PERIWOUND GRAY	44.25 5.51	64.75* 13.12	58.25 4.03	29.25 4.19	23.33* 3.33
PERIWOUND WHITE	38.25 2.39	70.50* 12.76	69.75* 8.38	37.50 2.84	27.33 1.20
WHITE MATTER	37.00 5.23	34.75 3.20	37.25 6.64	34.25 3.22	28.67 0.67
CAUDATE NUCLEUS	65.50 9.92	63.50 14.85	62.75 14.21	58.75 9.21	54.00 8.54
HIPPOCAMPUS	35.75 2.46	33.00 0.71	35.25 9.12	31.25 4.77	26.33 0.88
THALAMUS	64.25 3.30	56.00 8.55	56.25 8.42	51.50 3.10	48.67 4.41
HYPOTHALAMUS	33.25 8.37	35.00 7.11	32.50 10.?4	31.00 8.69	21.67* 1.45
RETICULAR FORMATION	61.00 5.96	63.75 1.49	63.50 9.99	56.50 5.01	48.33 4.33
BRAIN STEM &MEDULLA	33.50 3.77	40.75 6.05	36.25 7.03	31.25 5.68	26.67 2.60
CEREBELLUM GRAY	47.75 12.99	61.00 11.75	58.25 10.02	46.75 10.75	37.00 2.00
CEREBELLUM WHITE	45.25 10.38	47.50 6.96	53.50 11.20	46.00 9.55	38.67 3.71
TOTAL CBF	42.08 3.35	46.15 5.38	45.72 8.37	37.40 4.93	31.47* 1.05

Table 17: Changes in rCBF before (control) and at 5 min (n=8) and 20 min (n=3) after BMW in non-surviving cats. *Significant; $p<0.05$; control data are compared to post-wounding measurements. Mean ± SE, (ml/100g/min).

VARIABLES	CONTROL	5 min*	20 min	45 min	90 min
FRONTAL CORTEX	47.09 7.56	45.37 8.29	43.33 17.85		
PARIETAL CORTEX	51.47 8.33	39.87 7.21	40.67 13.68		
TEMPORAL CORTEX	30.50 4.37	36.81 6.19	35.33 8.84		
OCCIPITAL CORTEX	59.07 10.87	36.83 6.82	34.67 12.72		
PERIWOUND GRAY	39.51 6.17	25.61 5.46	20.33 14.84		
PERIWOUND WHITE	38.93 6.39	24.30 4.74	14.67 11.26		
WHITE MATTER	37.72 6.80	29.00 5.10	24.00 8.50		
CAUDATE NUCLEUS	68.28 10.42	56.43 11.14	43.33 14.75		
HIPPOCAMPUS	34.08 5.81	31.08 5.92	27.00 9.85		
THALAMUS	60.78 12.19	47.30 9.45	39.33 10.97		
HYPOTHALAMUS	29.37 5.23	25.90 5.84	25.33 12.33		
RETICULAR FORMATION	50.95 7.33	33.16 6.31	22.33* 3.18		
BRAIN STEM & MEDULLA	33.27 5.31	25.61 4.34	25.00 3.61		
CEREBELLUM GRAY	57.22 10.31	42.66 6.83	55.00 13.65		
CEREBELLUM WHITE	48.98 7.91	34.37 4.91	47.00 14.57		
TOTAL CBF	42.60 6.83	34.34 5.55	33.22 10.81		

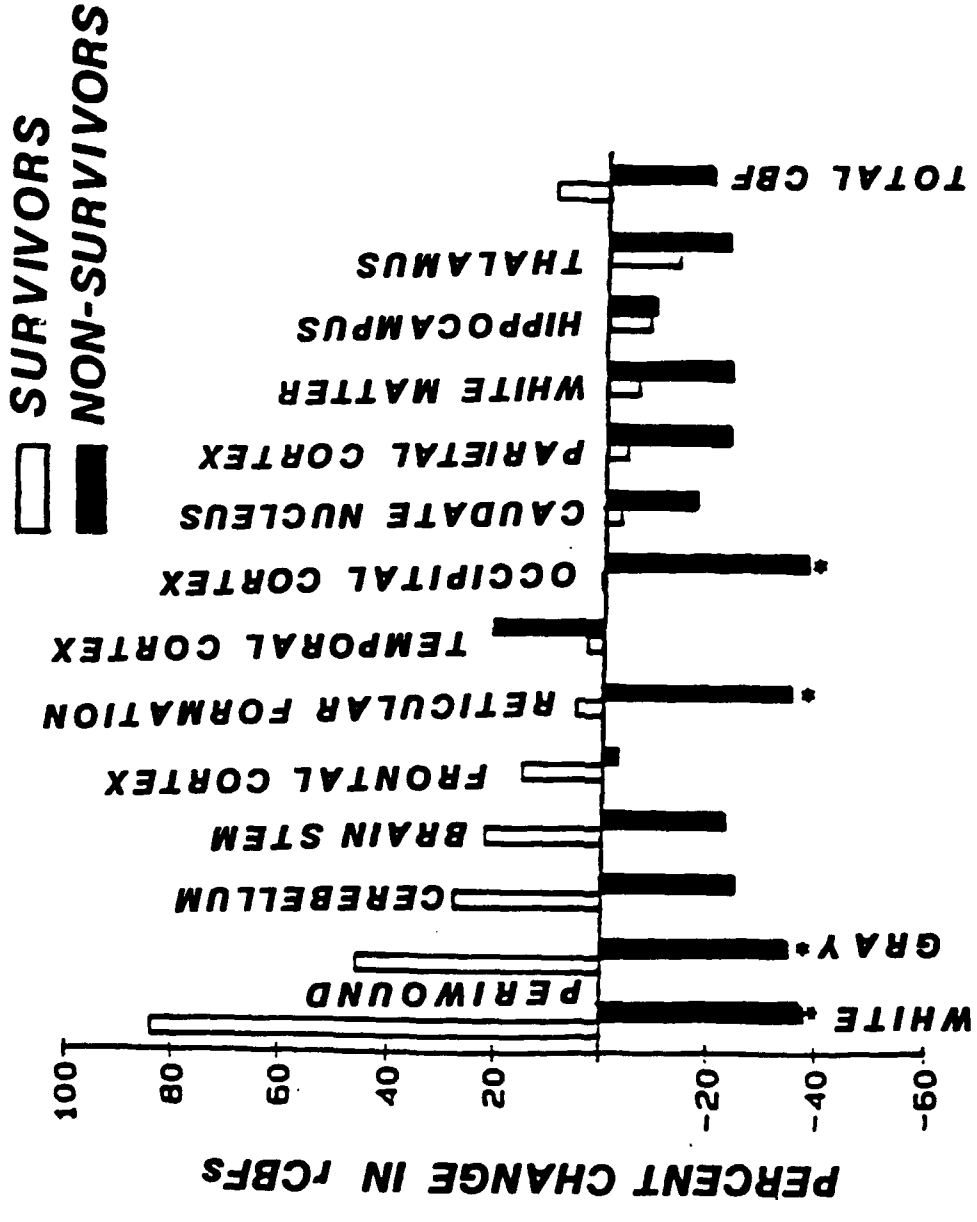


Figure 9: Comparison between percent change in rCBFs in surviving and non-surviving cats 5 min after wounding. In contrast to considerable reductions in the rCBF of periwound structures in non-survivors, the surviving cats showed significant hyperperfusion around the wound track. However, the residual total and rCBFs in non-survivors were theoretically adequate to allow autoregulation (i.e. >15 ml/100g/min) at this time point.
* significant; $p<0.05$, unpaired t-test.

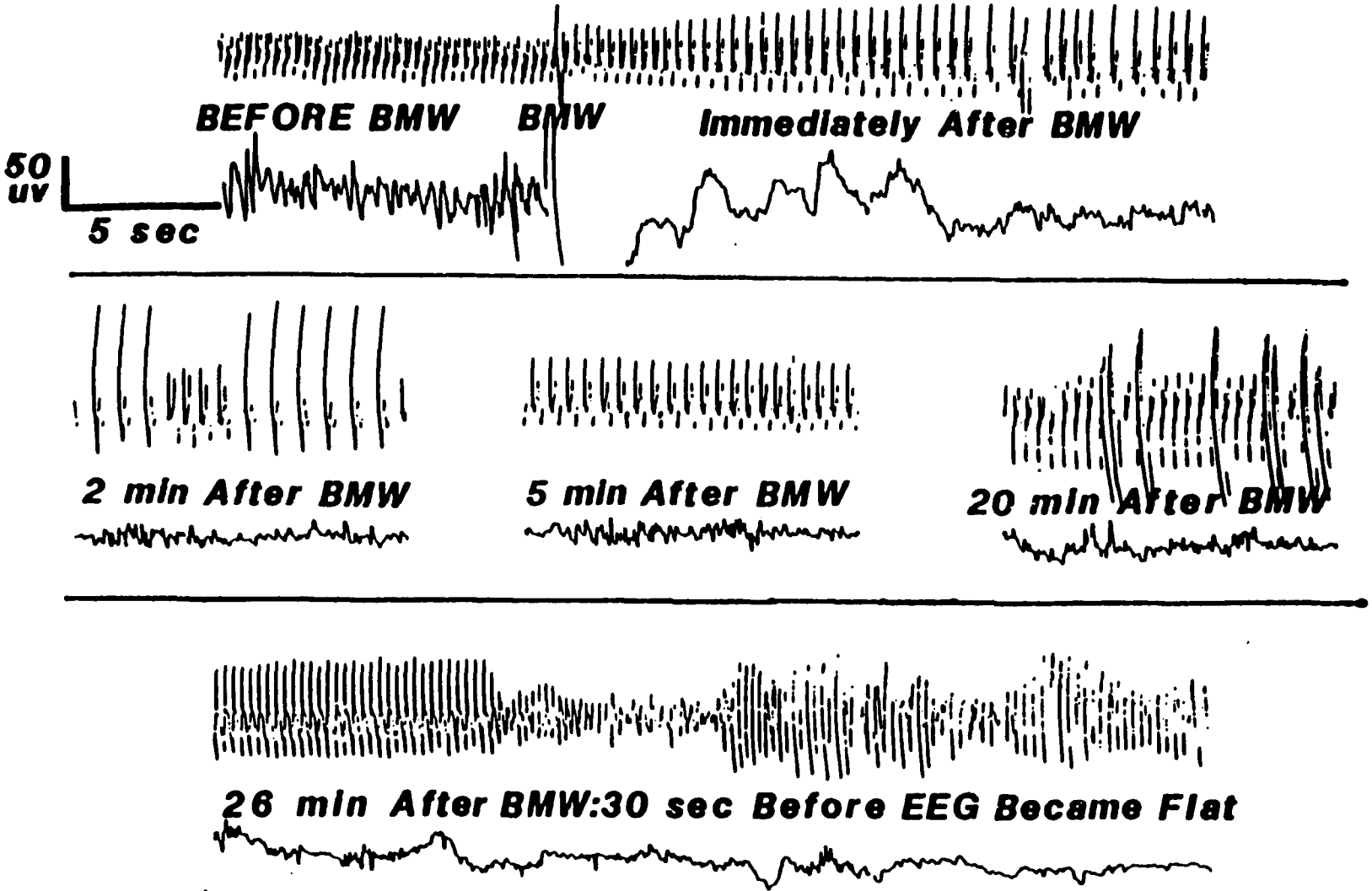


Figure 10: Changes in EEG and ECG following BMW in a non-surviving cat which apparently died from cardiac arrest. Note that both ECG arrythmias and reduced EEG activity actually improved 5 min after wounding. The ECG arrythmias reappeared 20 min post-wounding in face of a relatively reduced but otherwise normal EEG. Cardiac fibrilation in this cat (1/15) as shown at 26 min after wounding preceded the EEG failure.

Changes in MABP, ICP, CPP, CBF, CO, CO/Kg, total CBF, brain stem rCBF, (BSBF) arterial PO_2, PCO_2 and pH, in 5 non-surviving cats 2 to 6 minutes prior to death

The above data on 8 non-surviving cats includes some CO and brain stem blood flow (BSBF) measurements made up to 20 minutes before death. CO and CBF determinations made this far prior to death might not accurately reflect crucial CO, CBF and arterial blood gas and pH events close enough to death to allow satisfactory analysis of these important variables which might have contributed to death. Table 18 derived from Appendix 1A and 1B, provides physiologic data from 5 non-surviving cats which died no more than six minutes following a microsphere injection.

Minutes prior to death V was depressed in all animals causing decreased PaO_2, a $PaCO_2$ increase, and acidosis. While respiratory insufficiency was uniform, and ICP was highly elevated in 3, CPP markedly was diminished in 2 and CO was diminished in only 3. Only one cat exhibited a marked reduction in CBF despite a very adequate CPP. Any increase in CBF in response to elevated $PaCO_2$ appears to have been lost. One cat died from cardiac fibrillation despite an adequate CPP, CBF, CO and PaO_2.

Table 18: Mean Values Various Physiologic Variables in 4 Surviving Cats compared to Individual Variables in 4 Non-Surviving Cats 3-6 Minutes Prior to Death

	Survivors		Non-Survivors				
	Mean +SE		RI-11	RI-6	RI-4	RI-15	RI-13
Time of Variable After Wounding	5'	20'	5'	20'	5'	5'	20'
MABP(mmHg)	92 ±5	87 ±5	141	85	120	71	120
ICP (mmHg)	48 ±13	30 ±4	27	10	90	84	61
CPP (mmHg)	45 ±9	58 ±9	114	75	30	-13	59
BSBF (mmHg)	41 ±6	36 ±7	8	23	44	23	32
CO(ml/kg/min)	160 ±14	167 ±23	67	91	53	144	149
f/min	30 ±6	30 ±6	2	14	43	16	5
V(lit/min)	1.52 ±0.14	1.72 ±0.22	0.16	0.46	0.89	0.14	0.54
PaO_2 (mmHg)	115	116	40	-	47	56	72
$PaCO_2$ (mmHg)	34	32	58	-	71	52	52
pH	7.31	7.36	7.17	-	7.05	7.14	7.20
Mode of Death			Apnea	Apnea	Apnea	Apnea	cardiac arrest
Time of Death			9'	22'	8'	10'	26'

DISCUSSION

The systemic effects of CNS trauma have been known for over a century (28,50). Respiratory changes including apnea, systemic hypo- and hypertension, pulse changes and cardiac arrythmias arise from injury-induced paralysis of the brain stem 15,17,36,43,48) . With both closed and penetrating brain injury, brain stem perturbation arises as energy from an external source is applied to the brain. Energy deposited from closed head injury is directed centripitally towards midline brain structure (23), thus brain stem derangement and dysfunction would be expected. Brain stem effects (initial hypertension, bradycardia and respiratory slowing) have been documented in anesthetized monkeys and cats from missile wounds of various trjectories (7,13,24).

With both closed head injury and BMW energy is widely deposited throughout the brain. With closed head injury energy is deposited through the intact skull into the brain. With BMW, intracranial overpressures and intracranial pressure waves are set up from within the brain itself by missile passage which affects the brain at a distance particularly the brain stem. This distant pressure effects are quite apart from the local tissue laceration caused by the missile itself.

Because BMW is known to affect brain stem functions, we analyzed physiologic variables directly subserved by the brain stem (HR, MABP, f) to ascertain if any of these variables accounted for death after wounding. A detailed analysis of these functions as discussed below showed that after BMW the surviving cats had only a transient brain stem dysfunction, while non-survivors had manifestly unstable brain stem function (Figs 5 to 8). We also evaluated changes in intracranial and systemic variables after wounding: ICP, CPP, CBF, CO and V. Abnormalities in these parameters after BMW could also have lead to enhanced brain injury and death.

HEART RATE: The frequency and amplitude of HR fluctuations are determined by the autonomic control systems in the brain stem (56). HR changes accompany virtually every severe head injury producing usually an intense bradycardia which may be vagally mediated (17,20). Circulating catecholamines, which we have shown to be highly elevated following BMW (Soblosky et al, unpublished data) may have a direct effect on the myocardium (20,55).

In our experiments, initial bradycardia occurred in both surviving and non-surviving cats, but only survivors achieved a stable and normal HR a few min after wounding. Non-survivors on the other hand, continued to show an unstable HR until death indicating severe brain stem perturbation (Figure 7).

MEAN ARTERIAL BLOOD PRESSURE: The immediate elevation of MABP following experimental BMW as shown in our cats (Fig 5 and 6) has been well documented in monkeys as well (7,12,13). This response is similar to that observed

with non-penetrating head injury which also perturbs the brain stem (6,49,58,59).

After BMW the cat appears to maintain control of MABP better than the monkey. In our feline model, 20 min after BMW, MABP was close to control (+8%) while monkeys have been shown to exhibit -14% decrease 30 min post-wounding (12). Possibly this differing MABP response could represent experimental and anesthetic differences, but it could also indicate a true species difference in physiologic response. In our experiments the elevated MABP seen following wounding soon returned to control levels and remained steady in surviving cats. In non-survivors post-wounding MABP was variable and erratic indicating loss of medullary pressor control mechanisms.

RESPIRATORY FREQUENCY: Respiratory slowing and apnea are prominent feature of severe closed head injury and BMW (7,12,24,28,50). In our experiments all wounded cats exhibited a transient apnea of 5 to 70 sec immediately after wounding, followed by a decreased f. Cats that survived their brain wound rapidly regained a normal f and had no further bouts of transient apnea. Cats that ultimately succumbed on the other hand, had several transient apneas and continued to exhibit a severely reduced f, indicating loss of the brain stem and medullary respiratory control (Fig 8).

As has been intimated in the past the immediate brain stem effects of BMW and closed head injury (pressor response, bradycardia and respiratory dysfunction) are identical (6,15,44). This may imply that in so far as these immediate effects are concerned BMW and closed head injury may be considered equivalent injuries. What our present experiments seem to indicate is that fatally wounded cats exhibited immediate dysfunction in critical brain stem cardiorespiratory centers from which they never recovered. This would indicate a direct and instantaneous effect of kinetic energy transfer from missile to brain stem causing irreversible damage. Alternatively, it could imply a sequence of secondary intracerebral or systemic events which prevented the recovery of brain stem function seen in survivors.

ASSOCIATED INTRACRANIAL AND SYSTEMIC EVENTS FOLLOWING BMW: We evaluated increased ICP, reduced CPP, CBF and CO to see how these physiologic variables might have adversely affected the recovery of brain stem functions after BMW.

INTRACRANIAL PRESSURE: A BMW may cause an intracranial hematoma or increase cerebral blood volume from loss of autoregulatory control. Either of these mechanisms may actually elevate ICP and reduced CPP. Both an elevated ICP or reduced CPP may lead to brain stem dysfunction and apnea. In our experiments both the mean peak ICP overpressures following BMW and the later occurring static ICP pressure elevations 10 to 20 min after wounding were similar for the 4 surviving and 8 non-surviving cats. Likewise, CPPs in the two wounded subgroups were not markedly different. Though post-wounding ICPs and CPPs in both surviving and non-surviving cats were in the range where CBF regulation should occur: ICP < 70 mmHg; CPP> 40 mmHg (27,31,62), it would appear that

cerebrovascular regulatory mechanisms were particularly impaired in non-survivors as MABP fluctuations were more readily transmitted to the intracranial compartment in these animals (Fig 6).

Examination of ICP and CPP values in 5 cats 3 to 6 min prior to death (Table 18) reveals that ICP was markedly elevated in only 3, while CPP was markedly reduced in only 2. Thus, an elevated ICP or reduced CPP cannot solely account for the brain stem dysfunction and persistent cardiorespiratory abnormalities observed in fatally wounded cats.

The failure of ICP elevations or CPP reductions to explain persistent medullary dysfunction in our cats contrasts with results reported by Crockard et al (12,13). Their fatally wounded monkeys had an ICP of 75 mmHg 5 min after wounding while survivors had a mean ICP of 25 mmHg. Arguably high initial ICP in these animals damaged medullary cardiovascular control centers and this impairment was the basic neural flaw consequent to BMW. Alternative explanations must be sought in our model.

CHANGES IN CBF: Extreme reductions in CBF consequent to BMW or closed head injury have also been suggested as a major cause of death (6,14,24). In our experiments, though the mean total CBF was reduced in non-survivors as compared to survivors and the percent change in rCBFs 5 mins after wounding in non-survivors was markedly different from survivors (Fig 9), residual mean rCBFs including those in the brain stem were still much above the ischemic threshold of 15 ml/100g/min in the cat (29). Thus, fatal apnea cannot be ascribed to severely diminished brain stem blood flow.

Analysis of 5 individual non-surviving cats wherein rCBFs were measured 3 to 6 min prior to death (Table 18) reveals that only one animal developed severe brain stem ischemia. Despite a perfectly adequate CPP of 114 mmHg. brain stem blood flow fell to 8 ml/100g/min. Post-wounding CO in this animal fell to 67 ml/min/kg from a prewound level of 116 ml/min/kg. Possibly, the observed post-BMW decrease in CO coupled with a concomitant loss of CBF autoregulation caused the severe reduction in brain stem blood flow which, in turn, impaired medullary cardiorespiratory centers and caused apnea. Since this was the only cat out of 8 non-survivors which demonstrated severe post-wounding brain stem ischemia, one may conclude that cerebral ischemia did not cause fatal cardiorespiratory dysfunction in most fatally wounded cats.

Microsphere measure rCBFs in relatively large brain area. Possibly small, critical intramedullary nuclei did suffer ischemia and accounted for the respiratory and vasomotor dysfunction in non-surviving cats. Elevation of this possibility will have to wait future autoradiographic techniques for rCBF measurements in very small brain structures.

Though not related to post-wounding medullary dysfunction or immediate survival, an outstanding difference between survivors and non-survivors occurred in the blood flows of the periwound tissues. The periwound blood flow in survivors was significantly increased during the first 20 min after wounding some what reduced in non-survivors. Whether this difference in periwound blood flows indicate differences in CBF regulatory ability of survivors and non-survivors remains to be studied.

CHANGES IN CARDIAC OUTPUT: CO is a function of HR, myocardial contractillity, pressure within the aorta, and central venous pressure. Several of these factors are subject to reflex neurogenic control through brain stem autonomic nuclei which might be affected by BMW. While the literature is replete with investigations on the control of CO alone or on factors affecting CBF autoregulation, we have been able to find few studies which examine how CO might affect CBF. Davis and Sundt, (16) using normal pentobarbital anesthetized cats, concluded that CBF and CO are not interdependent but neither are they entirely independent of each other in the physiologic range. They observed that the brain maintained a constant CBF despite large increases in CO and suggested that this ability was part of the brain's autoregulatory ability.

Experimentally, only a few studies have examined CO immediately after brain injury (36). These investigators using primates showed that one min after severe BMW (80-100% mortality) CO had declined to about 60% of control and then remained stable at this level over the next 4 hours. This CO reduction was independent of ICP or CPP and occurred even after bilateral vagotomy. So excessive post-wounding parasympathetic activity affecting HR did not appear to be the underlying cause (13).

In our study the 8 non-surviving cats showed a 25% decrease in CO at 5 min after wounding. While suggestive, this decrease was not significant and by 20 min after wounding not even a suggestion of a CO decrease remained. Any post-wounding CO reductions which did occur probably did not occur consequent to medullary ischemia affecting reflex cardiovascular control center because our rCBF data indicate that brain stem medullary flow reductions are rare following BMW.

Among the 5 non-surviving cats which had COs determined 3 to 6 min prior to death, 2 had severe reduction (53-67 ml/min/kg) one had a moderate CO decrease (91 ml/min/kg), while 2 cats had quite normal post-wounding COs (144 to 149 ml/min/kg). These findings partially support the observation of Levett et al, (36) that BMW may result in a CO decrease but our data indicate that CO impairment does not appear to be a universal event following BMW nor may it be impuned as a unitary cause of post-wounding medullary dysfunction and death. The present results do suggest, however, that CO failure after BMW may be an important contributor to death if brain autoregulatory mechanisms also totally fail. In such an instance CBF would fall concomitantly with CO and global brain ischemia might result. Additionally, CO reductions consequent to BMW could decrease myocardial blood flow and cause death from cardiac dysfunction.

RESPIRATORY DYSFUNCTION IN FATALLY WOUNDED CATS: Although all brain wounded cats exhibited some degree of respiratory dysfunction, in non-survivors f became so reduced that compensatory increase in tidal volume could not improve the significantly reduced V (Table 15). Analysis of data presented in Table 7 indicates that all fatally wounded cats studied 3 to 6 min prior to death had significant decreases in V and 4 deaths could be ascribed to apnea. Cardiac arrest caused the remaining death. We infer that the persistently reduced V

in 3 of the 5 cats resulted from direct brain stem injury consequent to transfer of missile energy to medullary respiratory centers. In 2 others, V reductions are ascribed to secondary events affecting respiratory centers: brain stem ischemia and a markedly reduced CPP. Interestingly enough, post-wounding ventilation was most seriously affected in these two animals.

CARDIAC ARRHYTHMIAS: The cardiac arrhythmias that we observed in both surviving and non-surviving cats immediately after wounding occur not only after BMW but are seen also after closed head injury (6,8), and increased ICP (9,25). Medullary dysfunction consequent to BMW could have affected that heart directly through autonomic innervation. Myocadial damage and arrhythmias after brain injury in humans and animals have been documented and attributed to persisting high plasma catecholamine levels (8,25,42).

In our experiments in addition to early cardiac arrhythmias, a late cardiac arrhythmia accounted for death in one-non-surviving cat (Fig 10 and Table 18). This fatal cardiac dysfunction did not occur consequent to reduced V and hypoxia or from reduced CO, but could have resulted from cardiocmyopathic effects of excessive plasma catecholamines which have been shown to markedly increase after BMW (Soblosky et al, unpublished data).

CAUSES OF DEATH FOLLOWING BMW AND IMPLICATIONS FOR RESUSCITATION: In this feline model of brain wounding the immediate cardiorespiratory, brain stem effects of missile injury appears stereotyped and similar to that noted in many other species sustaining brain injury. Both penetrating and non-penetrating brain injury may cause an immediate, fatal apnea. The later occurring, associated intracranial and systemic events of BMW are quite variable, may be species specific, and are more often associated with missile injury than closed or percussion head injury. In the present model of brain wounding no one or two physiologic factors appeared to account for the appearance of sustained apnea after wounding. Rather, fatal apnea appeared to result from an interplay of many factors none of which occurred uniformly. These include indirect damage to brain stem respiratory centers from missile energy, loss of CBF autoregulation, CO decrease, cerebral ischemia, ICP increase or CPP decrease. After BMW death may not occur from an expected apnea but, rather from cardiac arrest.

While intracranial and respiratory factors are usually emphasized in the emergent treatment of the brain injured (28,44,45,50), the treatment of early cardiac dysfunction is less often considered, though it has been repeatedly documented (13,17,24). Our data as well as those of prior works (42) indicate that attention should be given to emergent care of cardiac dysfunction after brain wounding.

In our experiments cats which survived brain wounding appeared to maintain all aspects of physiologic function (HR, MABP, f, CBF, CO, V) while animals which died tended to exhibit multiple cardiorespiratory abnormalities.

Despite wounds of the same trajectory and energy some animals could regain normal medullary function and lived, while others could not and died. Factors accounting for the difference between these 2 groups should be sought because manipulation of critical factors may decrease mortality of brain wounds.

APPENDIX 1A

Physiologic Variables in Brain-Wounded, Spontaneously Breathing Cats: 4 Cats Survived Their Wound to 90'. 5 Cats Died. CO and CBF were Determined in These 5 Cats Within 5 Minutes of Death (s= survive; d= died; w= wound; MABP, ICP, CPP= mmHg; CO/Kg= ml/min/Kg; TCBF= total CBF, BSBF= brain stem blood flow; ml/100g/min)

		MABP	ICP	CPP	CO	CO/KG	TCBF	BSBF	Out-come
	R1-3	93	7	86	674	178	43	39	s 90'
Survivors	R1-5	65	10	55	371	124	42	33	s 90'
Control	R1-8	103	9	94	670	197	50	39	s 90'
(pre w)	R1-10	87	11	76	366	102	34	23	s 90'
	X	87	9	78	520	150	42	34	
	(±SE)	(±8)	(±1)	(±8)	(±22)	(±22)	(±4)	(±4)	
Non-	RI-4	97	9	88	428	138	58	40	d 8'
Survivors	RI-11	103	12	91	395	116	49	39	d 8'
Control	RI-15	78	2	76	355	134	50	40	d 10'
(pre w)	RI- 6	90	4	86	830	163	75	59	d 25'
	RI-13	89	1	88	333	70	17	11	d 26'
	X	91	6	86	468	124	50	38	
	(±SE)	(±4)	(±2)	(±3)	(±92)	(±16)	(±9)	(±8)	
Survivors	RI-3	107	80	27	689	184	51	55	s 90'
5 min	RI-5	82	41	41	481	160	42	38	s 90'
(post w)	RI-8	92	50	43	652	192	58	44	s 90'
	RI-10	88	19	69	469	130	33	26	s 90'
	X	92	48	45	573	160	46	41	
	(±SE)	(±5)	(±13)	(±9)	(±57)	(±14)	(±5)	(±6)	
Non-	RI-4	120	90	30	165	53	57	44	d 8'
Survivors	RI-11	141	27	114	228	67	11	8	d 8'
5 min	RI-15	71	84	-14	382	144	31	23	d 10'
(post w)	X	111	67	43	258	88	33	25	
	(±SE)	(±21)	(±20)	(±38)	(±65)	(±28)	(±13)	(±10)	
Non-	RI-6	107	26	81	697	137	43	37	d 25'
Survivors	RI-13	102	64	38	589	124	41	30	d 26'
5 min	X	104	45	60	643	130	42	34	
(post w)									
Survivors	RI-3	93	35	58	631	168	43	38	s 90'
20 min	RI-5	72	40	32	638	213	42	33	s 90'
(post w)	RI-8	93	24	69	-	-	69	54	s 90'
	RI-10	90	21	69	431	120	29	20	s 90'
	X	87	30	58	567	167	46	36	
	(±SE)	(±5)	(±4)	(±9)	(±59)	(±23)	(±8)	(±7)	
Non-	R1-6	85	10	75	493	97	23	23	d 25'
Survivors	R1-13	120	61	59	706	149	55	32	d 26'
20 min	X	102	36	67	600	123	39	28	
(post w)									

APPENDIX 1B

Respiratory Variables in Brain Wounded Spontaneously Breathing Cats: 4 Cats Survived Their Wound to 90'. 5 cats died. Respiratory and Blood Gas and pH Variables Within 3 to 5 Minutes of Death. (s= survive; d= died; w= wound; f= resp/ min, V_T= ml, V_I= l/min; PaO_2, $PaCO_2$= mmHg)

		f	V_T	V_I	PaO_2	$PaCO_2$	pH	Out-come
Survivors	RI-3	16	68	1.02	112	36	7.37	s 90'
Control	RI-5	40	45	1.82	123	35	7.34	s 90'
(pre w)	RI-8	26	79	2.07	107	27	7.44	s 90'
	RI10	35	46	1.67	115	27	7.35	s 90'
	X	29	60	1.64	114	31	7.37	
	(±SE)	(±6)	(±8)	(±0.22	(±3)	(±3)	(±0.02)	
Non-	RI-4	36	30	1.18	111	29	7.39	d 8'
Survivors	RI-11	20	67	1.25	108	34	7.35	d 8'
Control	RI-15	49	32	1.58	105	28	7.38	d 10'
(pre w)	RI-6	26	75	1.97	109	32	7.42	d 25'
	RI-13	20	80	1.60	115	33	7.34	d 26'
	X	30	57	1.52	110	31	7.38	
	(±SE)	(±6)	(±11)	(±0.14)	(±2)	(±1)	(±0.01)	
Survivors	RI-3	14	59	0.82	108	43	7.27	s 90'
5 min	RI-5	36	50	1.70	128	35	7.28	s 90'
(post w)	RI-8	25	27	2.07	106	28	7.38	s 90'
	RI-10	29	52	1.62	117	31	7.32	s 90'
	X	26	47	1.55	115	34	7.31	
	(±SE)	(±5)	(±7)	(±0.26)	(±5)	(±3)	(±0.02)	
Non-	RI-4	43	46	0.89	47	71	7.05	d 8'
Survivors	RI-11	2	80	0.16	40	58	7.17	d 8'
5 min	RI-15	16	32	0.14	56	52	7.14	d 10'
(post w)	X	20	53	0.40	48	60	7.12	
	(±SE)	(±12)	(±14)	(±0.25)	(±5)	(±6)	(±0.04)	
Non-	RI-6	28	78	1.75	76	39	7.30	d 25'
Survivors	RI-13	5	108	0.54	79	45	7.24	d 26'
5 min	X	16	93	1.14	42	42	7.27	
(post w)								
Survivors	RI-3	15	64	1.25	118	38	7.37	s 90'
20 min	RI-5	39	51	1.90	123	32	7.34	s 90'
(post w)	RI-8	43	49	2.26	107	27	7.38	s 90'
	RI-10	24	47	1.49	115	32	7.33	s 90'
	X	30	53	1.72	116	32	7.35	
	(±SE)	(±6)	(±4)	(±0.22)	(±4)	(±2)	(±0.01)	
Non-	R1-6	14	60	0.46	-	-	-	d 25'
Survivors	R1-13	4	154	0.47	72	52	7.20	d 26'
20 min	X	9	107	0.46	-	-	-	
(post w)								

APPENDIX 2A
Critical Physiological Variables in Brain Wounded Animals
(Pooled Data: Survivors Plus Non Survivors)

		Monkey (1.3 J brain wound through skull trephine)						Cat (1.4 J brain wound through intact skull) (Torbati)				
Variable	ref	control	PW time 1-5'	%	PW time 30'	%		control	PW time 1-5'	%	PW time 20'	%
HR	1	192	133	-31	192	0		207	164	-21	194	-6
	3	200	168	-16	178	-11						
MABP	1	90	96	+7	77	-14		83	97	+17	90	+8
	3	97	117	+21	75	-23						
f	1	32	28	-13	28	-13		29	20	-31	23	-21
ICP	1	1	42	-	31	-		7	46	-	30	-
	3	10	52	-	20	-						
CPP	1	89	54	-39	46	-48		76	51	-33	60	-21
	2	90	54	-40	58	-36						
	3	90	41	-54	60	-33						
CBF	2	43	26	-40	16	-63		42	38	-10	40	-5
	3	42	19	-55	16	-62						
CI	3	1.67	1.0	-40	1.0	-40	CO	127	111	-13	146	+15
Anesthesia: phencyclidine ± barbiturate								Anesthesia pentobarbital				

References
1. Crockard et al: Neurosurg 46:776, 1977
2. Crockard et al: Neurosurg 46:784, 1977
3. Levett et al: Surg Neurol 13:59, 1980

no respiratory support

HR= heart rate (beats/min); MABP= mean arterial blood pressure (mmHg);
f= frequency (respirations/min); ICP= intracranial pressure (mmHg);
CPP= cerebral perfusion pressure (mmHg); CBF= cerebral blood flow (ml/100g/min);
CI= cardiac index (ml/kg/min/sq m); CO= cardiac output (ml/kg/min)

APPENDIX 2B
Critical Physiological Variables in Animals Sustaining Percussion or Impact Injury

	Monkey Impact Injury[1]					Cat Fluid Percussion[2]				
Variable	control	PW time 1'	%	PW time 30'	%	control	PW time 3-15'	%	PW time 30'	%
HR	186	87	-53	170	-9	-	-	-	-	-
MABP	98	110	+12	92	-6	118	204		98	-17
f	-	-	-	-	-					
ICP	7	21	-	16	-	3	44	-	9	-
CPP	91	89	-22	76	-11	115	160	+40	89	-23
CBF	41	30	-27	27	-34	~31			29	-6
CI	1.04	0.30	-63	0.55	-47	-	-		-	-
Anesthesia: phencyclidine						anesthesia: methohexital + 60 % $N_2$0-40 % 0_2 paralyzed				

1 Brown and Brown: Arch Neurol & Psychiat

2 Saunders et al: J Neurosurg 51:18-26, 1979

HR= heart rate (beats/min); MABP= mean arterial blood pressure (mmHg)
f= frequency (respirations/min); ICP= intracranial pressure (mmHg)
CPP= cerebral perfusion pressure (mmHg); CBF= cerebral blood flow (ml/100g/min);
CI= cardiac index (ml/kg/min/sq m)

APPENDIX 2C

Critical Physiological Variables in Surviving and Non-Surviving Monkeys and Cats Receiving a Brain Wound

Monkeys
1.3J Brain Wound
(Crockard et al)

Survivors

Variable	control	PW time 1-5'	%	PW time 20'	%
HR	200	160	-20	170	-15
MABP	105	105	0	90	-14
f	30	28	-7	30	0
ICP	5	25	-	35	-
CPP	95	80	-16	55	-42
CBF	40	22	-45	25	-38

Cats
1.4J Brain Wound
(Torbati et al)

Survivors

variable	control	PW time 5'	%	PW time 30'	%
HR	222	174	-22	207	-7
MABP	87	92	+6	87	0
f	29	26	-10	30	+3
ICP	9	47	-	30	-
CPP	78	45	-42	57	-27
CBF	42	46	+10	46	+10
CO	150	167	+11	167	0

Monkey
1.3J Brain Wound
Non-Survivors

Variable	control	PW time 1-5'	%	PW time 20'	%
HR	200	90	-55	210	+10
MABP	105	100	-5	50	-52
f	30	12	-60	10	-67
ICP	5	75		30	-
CPP	95	25	-74	20	-78
CBF	40	30	-25	12	-70

Cat
1.4J Brain Wound
Non-Survivors

variable	control	PW time 5'	%	PW time 30'	%
HR	200	159	-20	176	-12
MABP	81	99	+22	94	+16
f	30	18	-41	13	-55
ICP	6	45	-	31	-
CPP	75	54	-28	63	-16
CBF	43	34	-19	33	-22
CO	115	83	-28	126	+10

Physiologic Variables abbreviations as per Appendix 2A

SECTION C: THE EFFECT OF BRAIN WOUNDING ON LUNG WATER:

BACKGROUND: Earlier experiences with missile wounding of the brain in our model (Final Summary Report, DAMD17-83-C-3145, 1987) and much neurosurgical literature indicate that there is a potent brain-lung axis and brain injury is frequently associated with decreased PaO_2 levels or frank neurogenic pulmonary edema (3,4,10,18,44,51). For this reason we evaluated lung water in a group of non-respirated cats after a brain missile wound. Cats used for these measurements were those used to study the effect of wounding on selected physiologic variables in spontaneously breathing, non- respirated cats in section B of this study.

METHODS: A few minutes after death in non-survivors and after euthanasia (lethal dose of pentobarbital) in survivors, the abdomen was opened and the descending aorta incised to drain blood for a few minutes. Then, following a midline sternotomy the entire lung was quickly removed. Contamination by residual blood from the severed vessels in the thoracic cavity was avoided. The trachea and large branches of the bronchial tree were removed and the isolated lungs were then placed in a tared container. The container was then reweighed and placed in 60°C oven. The lungs were dried to a constant weight over 48 to 72 hours. The percent water content of lungs was calculated as: wet weight- dry weight/wet weight X 100.

STATISTICAL ANALYSIS: Unpaired t-test

RESULTS: Some lungs of both unwounded and wounded cats had areas of hemorrhage in them. The lung water content of 3 unwounded cats was 71.7 $\pm$ 1.8%. Four wounded survivors had lung water contents of 71.8 $\pm$ 0.8% while non-survivors had 73.6 $\pm$ 1.1 % lung water. These differences were not significant.

DISCUSSION: Our results showing no signficant lung water gain following wounding are in contrast to those reported by Miller et al (44) who found significant increase in lung tissue wet-to-dry weight ratios (3.74 compared with a control of 2.92; $p < 0.001$) in cats following brain fluid percussion injury, in which 60% of their cats became premanently apneic within 6 min post injury. A considerable species variability also has been found in the amount of pulmonary edema seen after brain injury (5). For example, rats routinely develop a massive hemorrhagic pulmonary edema within a few minutes of a lethal, closed head injury (5). In contrast, brain injury in cat, dog, rabbit, guinea pig, baboon and chimpanzee has been shown to cause but little increase in the lung weight/body weight ratios (3,10).

REFERENCES FOR SECTIONS A,B, AND C

1. Artru AA (1987) Reduction of cerebrospinal fluid pressure by hypocapnia: Changes in cerebral blood volume, cerebrospinal fluid volume, and brain tissue water electrolytes. J Cereb Blood Flow Metabol 7:471-479

2. Bean JW, Lingnell J, Burgess DW (1972) Cerebral O_2, CO_2, regional cerebral vascular control and hyperbaric oxygenation. J Appl Physiol 32 650-657

3. Reckman DL (1978) Rat lung hyper-reactivity to stress. Aviat Space Environ Med 49:70-72

4. Beckman DL, Bean, JW, Baslock DR (1971) Sympathetic influence on lung compliance and surface forces in head injury. J Appl Physiol 30:394-399

5. Beckman DL, Bean JW (1969) Plumonary damage and head injury. Proc Soc Exp Biol Med 130:5-9

6. Brown, FD, Jafar, JJ, Krieger K, Johns L, et al 1982) Cardiac and cerebral changes following experimental head injury in: Head Injury: Basic and clinical aspect. R.G. Grossman and P.L. Gildenberg (eds.), Raven Press: NY, pp 151-157

7. Carey ME, Sarna GS, Farrell JB, Happel LT (1989) Experimental missile wound to the brain. J Neurosurg 71:754-764

8. Clifton GL, Robertson CS, Kyper K, et al (1983) Cardiovascular response to severe head innury. J Neurosurg 59:447-454

9. Clifton GL, Ziegler MG, Grossman RG (1981) Circulating catecholamines and sympathetic activity after head injury. Neurosurg 8:10-14

10. Crittenden DJ, Beckman DL (1982) Traumatic head injury and pulmonary damage. J Trauma 22:766-769

11. Czernicki Z, Jurkiewiez J, Kunicki A (1975) The effect of hypocapnia on normal and increased intracranial pressure in cats and rabbits. In: Intracranial Pressure II (Lunberg N, Ponten V, Brock M, eds.), New York, Springer-Verlag, pp 471-473

12. Crockard HA, Brown FD, Johns LM, Mullen S (1977) An experimental cerebral missile injury in primates. J Neurosurg 46:776-783

13. Crockard HA, Brown FD, Calica AB, Johns LM, Mullen S (1977) Physiological Consequences of experimental missile injury and use of data analysis to predict survival. J Neurosurg 46:784-794

14. Crockard, HA, Brown FD, Trimble J and Mullan S (1977) Somatosensory evoked potentials, cerebral blood flow and metabolism following cerebral missile trauma in monkeys. Surg. Neurol. 7:281-287

15. Crockard HA, Johns LM, Levett JM, et al. (1979) "Brain stem" effects of exerpimental cerebral missile injury. In "Neural Trauma (Popps AJ, ed.), New York, Raven Press, pp 19-25

16. Davis DH and Sundt TM (1980) Relationship of cerebral blood flow to cardiac output, mean arterial pressure, blood volume and alpha and beta blodkade in cats. J Neurosurg 52:745-754

17. Denny-Brown D, Russel WR (1941) Experimental cerebral concussion. Brain 64:93-164

18. Ducker TB, Simmons RL (1968) Increased intracranial pressure and pulmonary edema. The hemodynamic response of dogs and monkeys to increased intracranial pressure. J Neurosurg 28:118-123

19. Dumke PR, Schmidt CF (1943) Quantitative measurement of CBF. Am J Physiol 138:421-431

20. Evans DE, Alter WA, Shatsky SA, Gunby EN (1976) Cardiac arrhythmias resulting from experimetnal head injury. J Neurosurg 45:609-616

21. Fitch W, McDowall DG (1977) Systemic vascular responses to increased intracranial pressure. 1. Effects of progressive epidural balloon expansion on intracranial pressure and systemic circulation. J Neurol Neurosurg Psychiat 40:833-842

22. Fitch W, McDowall DG, Keaney NP, Pickerodt VWA (1977) Systemic vascular response to raised intracranial pressure. 2. The "Cushing" response in the presence of intracranial space-occupying lesions: systemic and cerebral haemodynamic studies in the dog and the baooon. J Neurol Neurosurg Psychiat 40:843-852

23. Gennarelli TA, Segwa H, Wald U, et al (1982) Physiological response to angular acceleration of the head, in: Head Injury: Basic and clinical aspects. R.G. Grossman, and P.L. Gildenberg (eds). Raven Press: NY, pp 129-140

24. Gerber AM, Moody RA (1972) Craniocerebral missile injuries in the monkey: an experimental physiological model. J Neurosurg 36:43-49

25. Graf CJ, Rossi NP (1978) Catecholamine response to intracranial hypertension. J Neurosurg 49:862-868

26. Granholm L, Siesjo BK (1969) The effects of hypercapnia and hypocapnia upon the cerebrospinal fluid lactate and pyruvate concentrations and upon the lactate, pyruvate, ATP, ADP, phosphocreatine and cretine concentrations of cat brain tissue. Acta Physiol Scand 75:257-266

27. Grubb RL, Raichle ME, Phelps ME, et al (1975) Effects of intracranial pressure on cerebral blood volume, blood flow, and oxygen utilization in monkeys. J Neurosurg 43:385-398

28. Horsley V (1894) The destructive effects of small projectiles. Nature 50:104-108

29. Hossmann KA (1982) Treatment of experimental cerebral ischemia. J Cereb Blood Flow Metab 2:275-297

30. James HE, Langfitt TW, Kumar VS (1976) Analysis of the response to therapeutic measures to reduce ICP in head injured patients. J Trauma 16:437-441

31. Johnston IH, Rowan JO, Harper AM et al (1973) Raised ICP and CBF II. Supratentorial and infratentorial mass lesions in primates. J Neurol Neursourg Psychiat 36:161-170

32. Kety SS, Schmidt CF (1946) The effects of active and passive hyperventilation on cerebral blood flow, cerebral oxygen consumption consumption, cardiac output, and blood pressure of normal young men. J Clin Invest 25:107-119

33. Kety SS, Schmidt CF (1948) The effects of altered arterial tensions of CO_2 and oxygen on cerebral blood flow and cerebral oxygen consumption of normal young men. J Clin Invest 27:484-492

34. Lambertsen CJ, Kough RE, Cooper DT, et.al. (1953) Oxygen toxicity. Effects in man of oxygen inhalation at 1 and 3.5 atmospheres upon blood gas transport, cerebral circulation and cerebral metabolism. J Appl Physiol 5:471-486

35. Langfitt TW, Gennarelli TA (1982) Can the outcome from head injury be improved? J Neurosurg 56:19-25

36. Levett JM, Johns LM, Replogle RL, et.al. (1980) Cardiovascular effects of experimental cerebral missile injury in primates. Surg Neurol 13:59-64

37. Levison M, Trunkey DD (1982) Initial assessment and resuscitation. Surg Clin North Amer 62:9-16

38. Lundberg N, Kjallquist A, Bien C (1959) Reduction of increased intracranial pressure by hyperventilation. Acta Psychiatr Scand (Suppl) 139:1-64

39. McDowall DG (1966) Interrelationship between blood oxygen tensions and cerebral blood flow. In: Oxygen measurements in blood and tissues (Payne JP, Hill DV, eds.), London, J and A Churchill, pp 205-219

40. McHenry LC, Slocum HC, Bivens HE, et.al. (1965) Hyperventilation in awake and anesthetized man: Effects on cerebral blood flow and cerebral metabolism. Arch Neurol 12:270-277

41. McKowen CH, Farrell JB, Carey ME (1989) rCBF following an experiemtnal missile wound to the brain. In: Intracranial Pressure VII (Hoff JT, Betz AL, eds.), Berlin, Springer-Verlag, pp 620-622

42. McLeod AA, Neil-Dwyer G, Meyer CHA, et.al. (1982) Cardiac sequelae of acute head injury. Br Heart J 47:221-226

43. Meyer JS, Kondo A, Nomura F, et.al. (1970) Cerebral hemodynamics and metabolism following experimental head injury. J Neurosurg 32:304-319

44. Millen JE, Glauser FL, Zimmerman M (1980) Physiological effects of controlled concussive brain trauma. J Appl Physiol 49:856-862

45. Miller JD, Becker DP (1982) Secondary insults to the injured brain. J Royal Coll Surg Edinburgh 27:292-298

46. Miller JD, Ledingham IM (1971) Reduction of increased intracranial pressure: Comparison between hyperbaric oxygen and hyperventilation. Arch neurol 24:210-216

47. Nakajima S, Meyer JS, Amano T, et al (1983) Cerebral vasomotor responsiveness duirng 100% oxygen inhalation in cerebral ischemia. Arch Neurol 40:271-276

48. Ommaya AK, Rockoff SD, Baldwin M (1964) Experiemental concussion. J. Neurosurg 21:249-265

49. Papo I, Caruselli G (1978) Effects on ICP of stopping controlled ventilation. Neurochirurgica 21:157-163

50. Polis A (1884) Recherches *experimentales sur* la commution cerebrale. Rev de Chir 273:645-655

51. Popp A, Shah D, Berman R (1982) Delayed pulmonary dysfunction in head injured patients. J Neurosurg 57:784-790

52. Raichle ME, Posner JB, Plum F (1970) Cerebral blood flow during and after hyperventilation. Arch neurol 23:394-403

53. Revich M (1964) Arterial PCO_2 and cerebral hemodynamics. Am J Physiol 206:25-35

54. Risberg J, Ancri D, Ingvar DH (1968) Regional cerebral blood volume changes related to blood flow variations. Scand J Clin Lab Invest (Suppl) 102:PXLC

55. Rosner MJ, Newsome HH, Becker DP (1984) Mechanical brain injury: The sympathoadrenal response. J Neurosurg 61:76-86

56. Saul JP (1990) Beat to beat variations of heart rate reflect modulation of cardiac autonomic outflow. NIPS 5:32-37

57. Saunders ML, Miller JD, Stablein D et al (1979) The effects of graded experimental trauma on cerebral blood flow and responsiveness to CO_2. J Neurosurg 51:18-26

58. Sullivan HG, Martinez J, Becker DP et al (1976) Fluid percussion model of mechanical brain injury in cat. J Neurosurg 45:520-534

59. Torbati D, Farrell JB, Davidson JF, et al (1989) Effects of hemorrhagic hypotension on CBF and intracranial pressure after brain milile wounding in anesthetized paralyzed cats. In: Intracranial Pressure VII (Hoff JT, Betz AL, eds.), Berlin, Springer-Verlag, pp 657-659

60. Weiner DE, Verrier RL, Miller DT et al (1967) Effect of adrenalectomy on hemodynamics and regional blood flow in the cat. Am J Physiol 213:473-476

61. Whal M, Deetjen P, Thurau K, et al (1970) Micropuncture evaluation of the importance of perivascular pH for the arteriolar diameter on the brain surface. Pflugers Arch 316:152-163

62. Zierski J (1987) Blood flow in brain structures during increased ICP. Acta Neurochirurgica (Suppl) 40:95-116

Section D: Effects of Increased Intracranial Pressure on Brain Biogenic Amines

INTRODUCTION

Missile wounding of the brain is associated with direct effects of missile damage since the damaging missile crushes tissues in its path. Missile wounding also causes indirect alterations of the brain many centimeters from the actual missile path itself. So-called "brain stem" effects of a cerebral hemisphere missile wound are prime examples of such an indirect effect. The "brain stem" effect is manifested by cardiovascular and respiratory abnormalities. Finally, missile wounding is accompanied by a transient, high increase in intracranial pressure (ICP) followed by a static ICP increase of lower magnitude. An ICP increase alone may be expected to alter brain function.

Our prior experiments have shown that immediately after wounding in the right cerebral hemisphere by a 2 mm 31 mg steel sphere, cats exhibited a marked, transient elevation of mean arterial blood pressure (MABP) with slight bradycardia (i.e. Cushing pressor response). They usually also have a depressed respiratory frequency. At lower missile energies the MABP pressor response is dominant; at higher missile energies the pressor response, while present, is overshadowed by respiratory depression which may take the form of fatal apnea.

This study focuses on the pressor response following brain wounding because it is so prominent with a missile injury of the brain and because it occurs with many other neurosurgical conditions accompanied by increased ICP. While the pressor response following increased ICP has been studied before (28,29,56,107,115,129), our present experiments evaluated biogenic amine changes in brain stem cardiovascular centers consequent to ICP increases. Such a study has not been undertaken before. Our ultimate goal is to evaluate brain stem pressor responses consequent to brain missile wounding (BMW). Since BMW entails a degree of increased ICP, our present studies on brain stem biogenic amines alterations following ICP elevations alone coupled with future studies on biogenic amine changes after BMW will help distinguish which biogenic amine changes observed after BMW arise from the wound itself and which may be attributed to increased ICP alone.

MATERIALS AND METHODOLOGY

A. General

Animals were first anesthesized with pentobarbital (40 mg/kg,IP). Adequacy of anesthesia was evaluated by cessation of limb withdrawal from thumb and index finger pinch between the toes and abolition of corneal reflex elicited by the touch of a paper wisp to the cornea. Arterial and venous cannulae were implanted in the right rear leg after treatment of the incision area with topical anesthetic (2% lidocaine). An

endotracheal tube, smeared with topical anesthetic (2% xylocaine jelly) was inserted after application of local anesthetic (.5 ml 2% xylocaine) to the epiglottis. The cat was then mounted in the stereotaxic frame and a mean arterial blood pressure (MABP) transducer attached to the arterial cannula for physiograph recording (Narco). Expired CO_2 was measured by an end tidal CO_2 monitor and also recorded on the physiograph. The depth of anesthesia was rechecked frequently during the experimental period and prior to sacrifice using the above criteria as well as the MABP and the respiratory rate. If required, the cats were given supplemental anesthesia (pentobarbital, 6.5 mg, I.V.). The head was partially shaved and a 5 cm. scalp incision made. A small burr hole (1.5 mm.) was placed in the skull and the dura excised for the insertion of an ICP transducer (Camino).

In order to increase ICP a 20 ga. spinal needle was inserted into the cisterna magna. The needle hub was connected by a length of rubber tubing to the outlet of a 2 liter aspirator bottle containing 1500 ml. mock CSF. Pressure in this mock CSF reservoir was raised by increasing air pressure within the sealed aspirator bottle by means of a 60 ml air filled syringe connected to the CSF reservoir bottle with appropriate tubing and fittings. By this means the ICP could be increased to 140 mm Hg within 30 seconds and maintained at any desired pressure for 6 minutes. In these experiments the ICP was rapidly raised to 120-140 mm Hg, maintained for 6 minutes and then the animals were sacrificed by decapitation. A rapid increase in ICP to 120-140 mm Hg was necessary to elicit the Cushing response. If apnea occurred consequent to the ICP increase and lasted more than 30 secs. the cat was attached to a respirator (Harvard Apparatus) and artificially ventilated. Control cats were treated in the exact same manner, but their ICP was not raised.

CHEMICALS and REAGENTS

Norepinephrine bitartrate (NE), epinephrine bitartrate (EPI), dopamine hydrochloride (DA), 5-hydroxytryptamine creatinine sulfate (5-HT, serotonin), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxyindoleacetic acid (5-HIAA), normetanephrine HCl (NM), monochloroacetic acid, ethylenediaminetetracetic acid (EDTA) and sodium hydroxide (NaOH) were purchased from Sigma Chemical Comp., St. Louis, MO. Sodium octyl sulfate was purchased from Eastman Kodak Co., Rochester, NY. The perchloric acid ($HClO_4$) and acetonitrile were obtained from J.T. Baker, Phillipsburg, NJ. All water was double distilled and deionized prior to use. Reference standard solutions of NE, EPI, DA, 5-HT, DOPAC, HVA, 5-HIAA and NM were made using .05 M perchloric acid containing .1 mM EDTA and stored at -70 °C. Working standard solutions (.5 or 1 pmol/µl) were prepared by serial dilutions using the same diluent and stored at 4 °C.

TISSUE DISSECTION

After rapid decapitation, the cranium was opened and the brain removed and frozen by immersion in cold dichlorodifluoromethane (-40 °C), then stored at -70 °C. until the sampling process. The euthanization, brain removal and freezing procedure was kept as uniform as possible and took approximately 18 minutes to complete for each animal. The brains were subsequently brought to approximately -15 °C and sliced into 3-5 mm thick coronal sections with reference to standard cat brain atlases (11,119). Each brain was completely sliced in one session (10 min). Tissue samples were taken from still frozen slices with the aid of tissue micropunches (1-1.5 mm dia.) or a scalpel, placed in dry ice and then stored at -70 °C until assayed. The brain stem was sliced in three 5 mm intervals, rostrally, starting at the obex. Two samples (left and right) of the nucleus tractus solitarius (NTS) and area A1C1 were taken from the caudal slice (see Fig 1, P 12). Two raphe nuclei samples were taken from the middle slice (see Fig. 1, P 8) and two raphe nuclei and two (left and right) locus coeruleus (LC) samples were taken from the rostral slice (see Fig 1., P 2). Hypothalamic slices were made by a three-bladed (3 mm interval) apparatus with the middle blade being positioned in the middle of the pituitary stalk. The anterior hypothalamus (AH) was dissected from the anterior slice by removing a 3 x 5 mm wide sample from the caudal midline. The posterior hypothalamus (PH) was sampled by removing a 4 x 4 x 4 mm triangular sample from the caudal midline of the posterior slice.

SAMPLE PREPARATION

Each of the left and right side samples of the NTS, Area A1C1 and LC were analyzed irdividually, but the four raphe nuclei samples were analyzed as one sample. Tissue samples were homogenized by ultrasonic disruption in .05 M $HClO_4$ containing .5 mM EDTA and 20 pmol of normetanephrine as an internal standard. The volume of .05 M $HClO_4$ used was optimized from preliminary tests so that an adequate signal would be output to a strip chart recorder set at 20 nA/V full scale. The volume of .05 M $HClO_4$ were as follows: 200 µl for the NTS, area A1C1 and the LC, 300 µl for the raphe N.; 1000 µl for the anterior and posterior hypothalamus. Following centrifugation (40,000 x g, 25 min.), the supernatant was removed and refrigerated until injection into the HPLC; 20 µl was injected into the HPLC. The remaining tissue pellets were solubilized in .2 M NaOH and assayed for protein content utilizing BCA* protein assay reagent (Pierce Chemical, Rockford, IL) using bovine serum albumin as a standard.

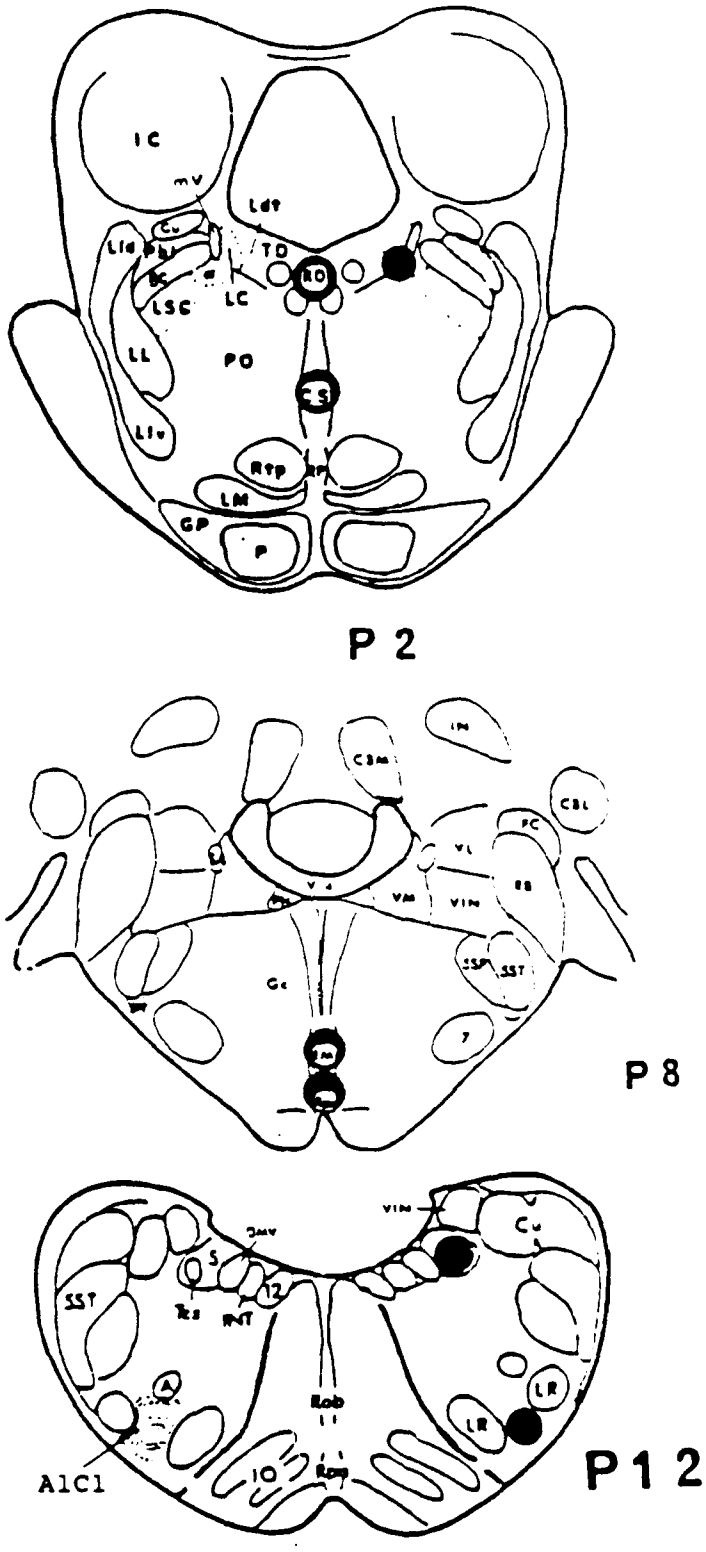


Fig. 1. LOCATION OF BRAINSTEM AREAS SAMPLED

HIGH PRESSURE LIQUID CHROMATOGRAPHY

The chromatograph utilized was a BAS 200 HPLC system with an electrochemical detector. Data was collected, integrated and quantified utilizing a PC-based chromatographic control system (BAS PC-II) (Bioanalytical Systems, W. Lafayette, IN.). The analytical column used was a 100 mm x 3.1 mm , BAS Phase II, 3 µm reverse phase column. The column was kept at 40 °C during its operation. The mobile phase consisted of .13 M monochloroacetic acid with .67 mM EDTA, adjusted to pH 3.1 with 10 M NaOH. This solution was filtered through .45 um durapore filter (Millipore, Bedford MA.) Sodium octyl sulphate was added as the ion pairing reagent at 170 mg/l and acetonitrile was added to a final concentration of 2.5%. The mobile phase was pumped at 1 ml/min and kept at 35 °C to prevent any possible outgassing. The detector potential was maintained at +800 mV vs. Ag/AgCl reference electrode. These chromatographic conditions permitted the routine quantification of NE, EPI, DA, 5-HT, DOPAC, HVA and 5-HIAA. within 18 minutes.

DATA ANALYSIS

Digitized data were used in order to quantify each compound. Concentrations of each compound were determined by comparing sample peak areas with peak areas obtained from a mixture of working standards. All measures of content were corrected by the normetanephrine recovery value for each sample. Concentrations are expressed as ng/mg protein.

Comparisons between the control and experimental groups were made using Analysis of Variance (ANOVA) contained in the SAS statistical package.

RESULTS

The effects on MABP and cerebral perfusion pressure (CPP; CPP=MABP minus ICP) resulting from increasing the ICP enough to elicit the Cushing response are displayed in Fig. 2 and Tables 1-3. The increased and sustained (6 min.) ICP elevation resulted in a concomitant and sustained increase in MABP in order to maintain adequate CPP (>40 mm Hg) to the brain.

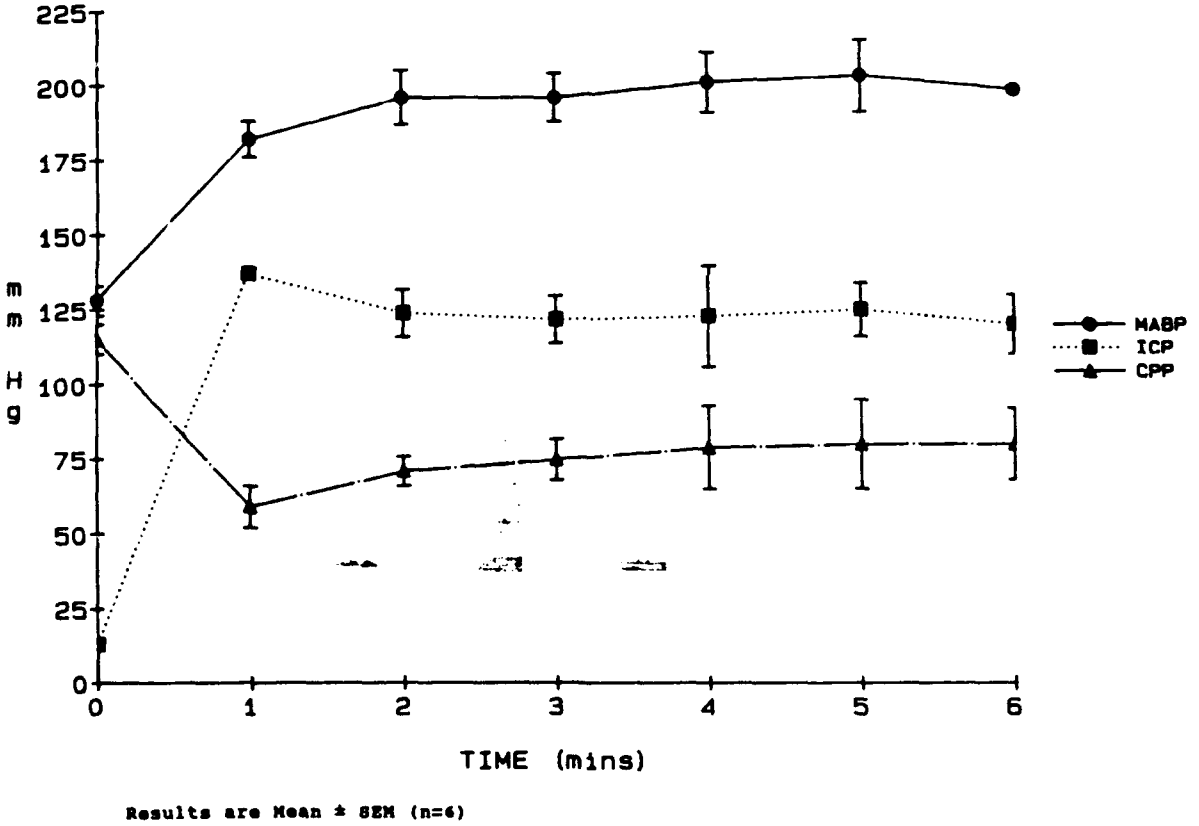


Fig. 2. MABP-ICP-CPP RELATIONSHIP IN CATS WITH INCREASED ICP

The results from cats which were surgically prepared in the same manner, except that the ICP was not raised, are displayed in Fig. 3 and Tables 1-3. This control group verified that the surgical preparation alone did not did not affect the MABP, CPP or ICP.

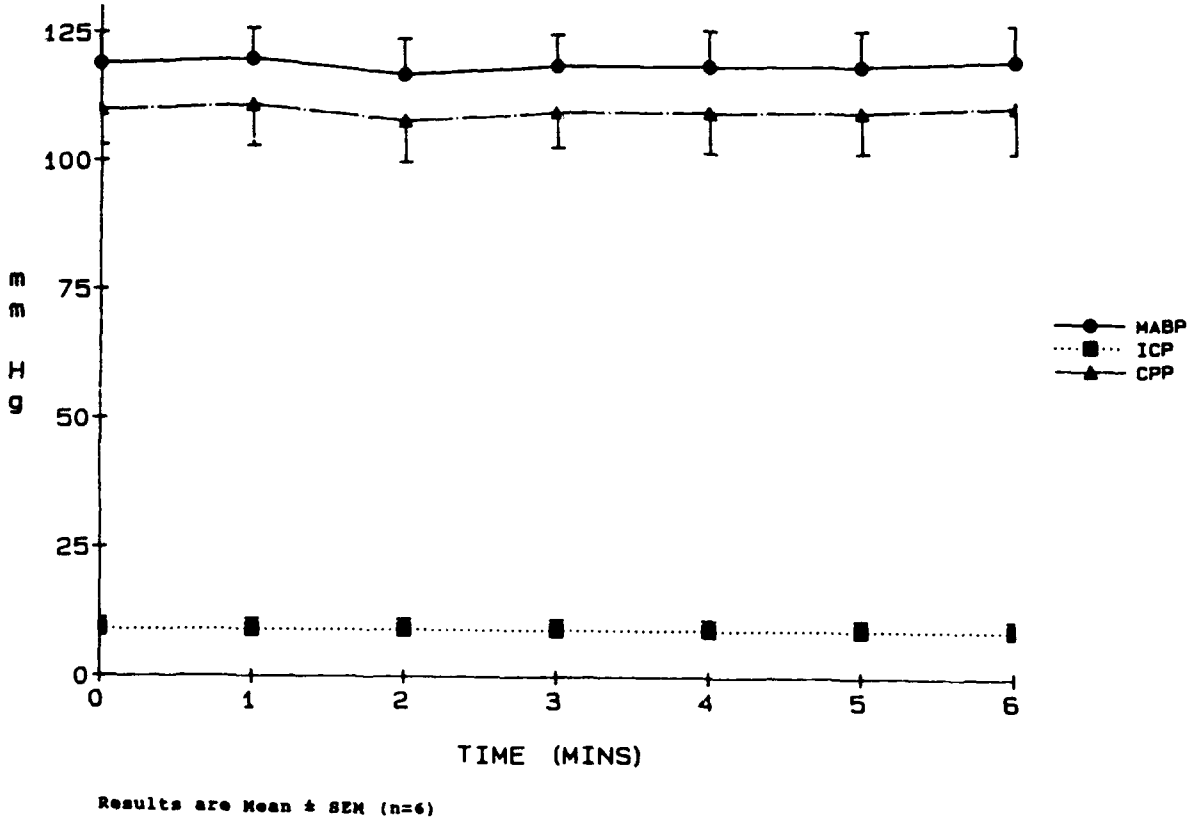
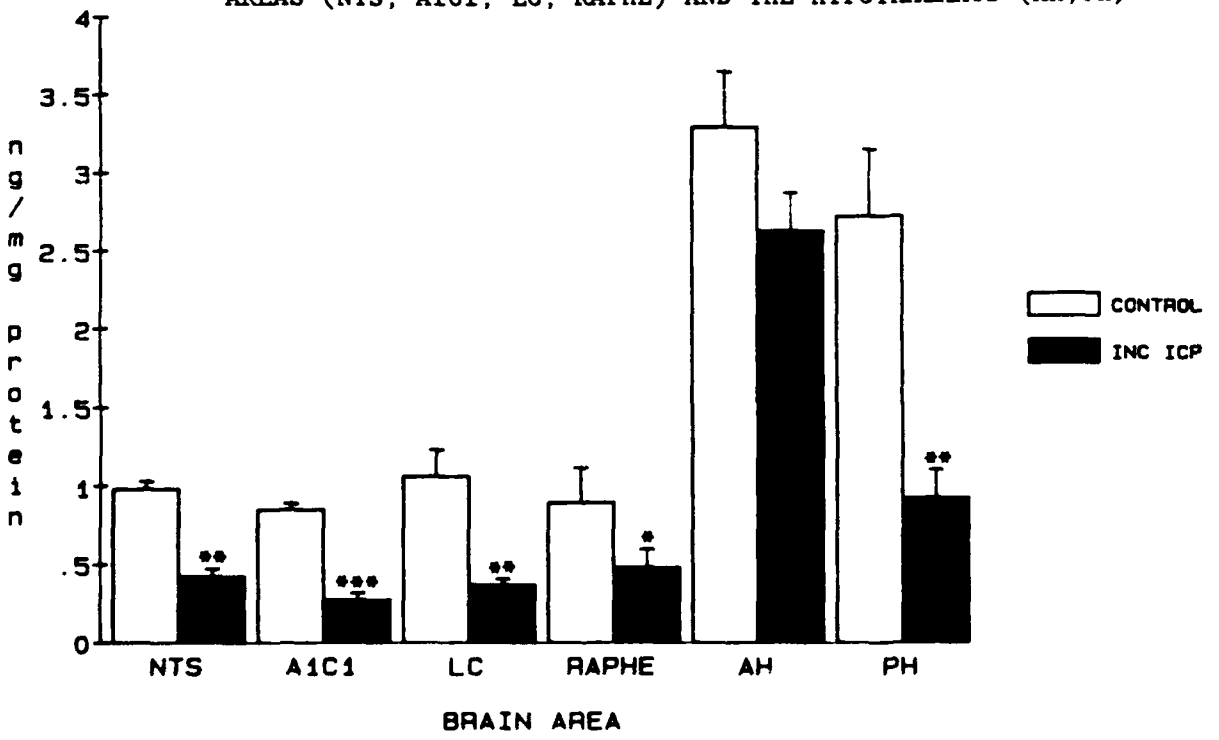


Fig. 3. MABP-ICP-CPP RELATIONSHIP IN CONTROL CATS (NO INCREASED ICP)

Statistical analyses were initially performed comparing the left and right side samples of the NTS, A1C1 and the LC in the control and experimental groups. After verifying that there were no differences due to sample side ($p > .8$), the data from the left and right side of each area was averaged and reanalyzed.

EPI was significantly decreased in the NTS (57%, $p < .01$), area A1C1 (68%, $p < .001$), LC (66%, $p < .01$), raphe nuclei (47%, p,.05) and PH (66%, $p < .01$) as a result of increased ICP (Fig. 4; Tables 4-7,9).

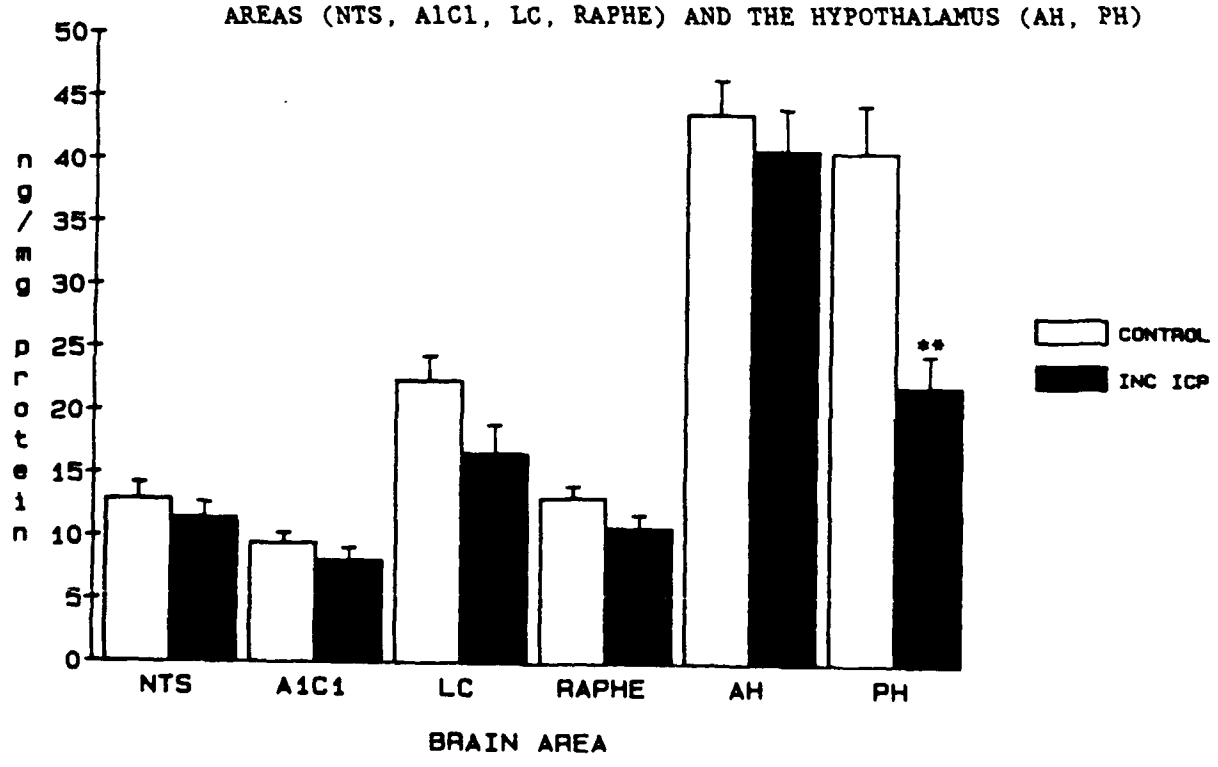
Fig. 4. EFFECTS OF INCREASED ICP ON EPINEPHRINE (EPI) IN BRAINSTEM AREAS (NTS, A1C1, LC, RAPHE) AND THE HYPOTHALAMUS (AH, PH)



Results are Mean +/- SEM (n=6). * P<.05, ** P<.01, *** P<.001

NE was a significantly decreased by 46% in the PH consequent to increased ICP ($p<.01$). Decreases in NE also occurred in the NTS (12%), area A1C1 (14%), LC (26%) and AH (16%) but they did not reach statistical significance (Fig. 5; Tables 4-6,8,9).

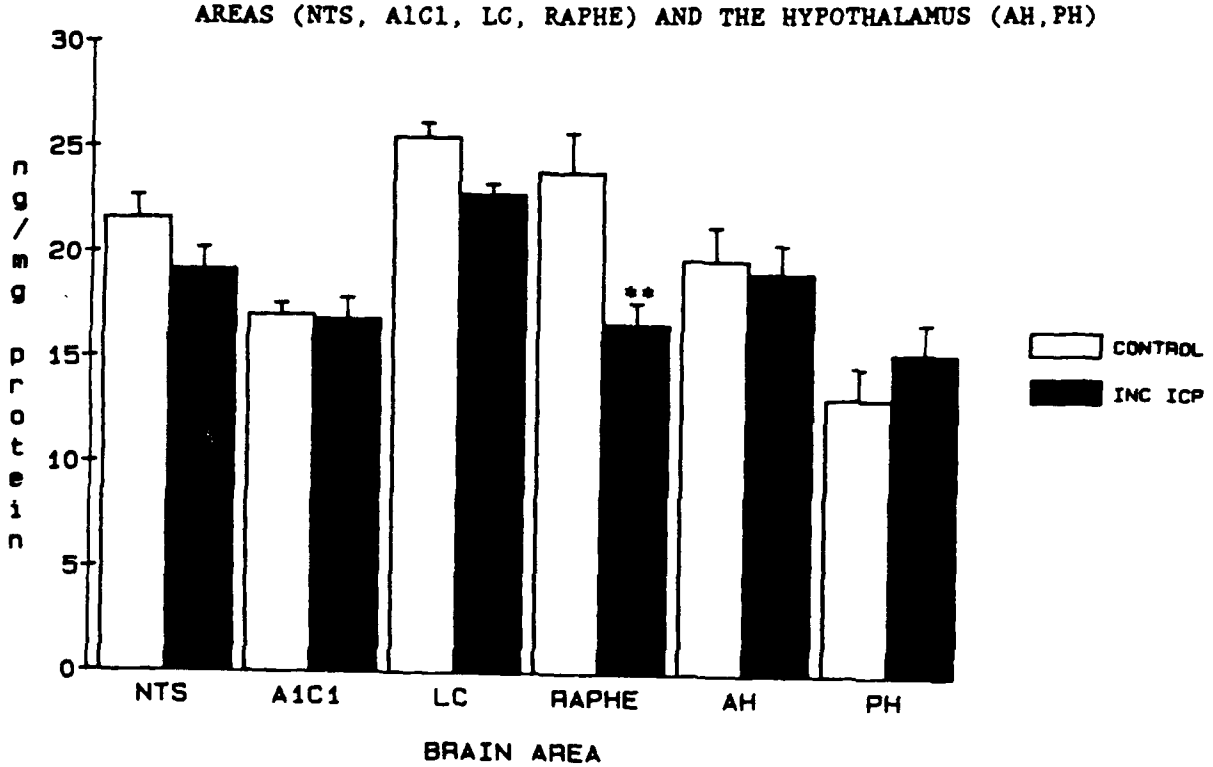
Fig. 5. EFFECTS OF INCREASED ICP ON NOREPINEPHRINE (NE) IN BRAINSTEM AREAS (NTS, A1C1, LC, RAPHE) AND THE HYPOTHALAMUS (AH, PH)



Results are Mean +/-SEM (n=8). ** P<.01.

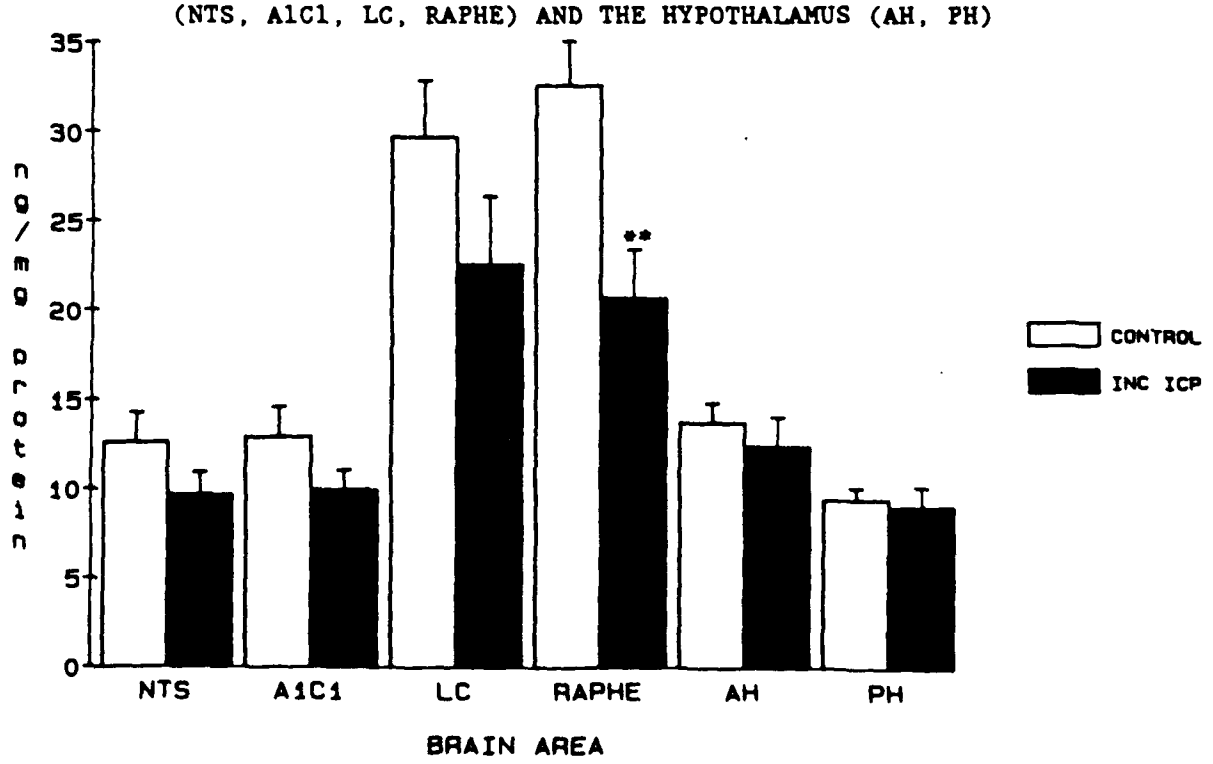
5-HT and its metabolite, 5-HIAA, were significantly decreased in the raphe nucleus by 30% and 36% respectively as a consequence of increased ICP (both $p<.01$). But the 5-HT/5-HIAA ratio (.81) was not significant when compared to the control data (.73) (Figs. 6,7; Table 7).

Fig. 6. EFFECTS OF INCREASED ICP ON SEROTONIN (5-HT) IN BRAINSTEM AREAS (NTS, A1C1, LC, RAPHE) AND THE HYPOTHALAMUS (AH,PH)



Results are Mean +/- SEM, (n=6), ** p<.01

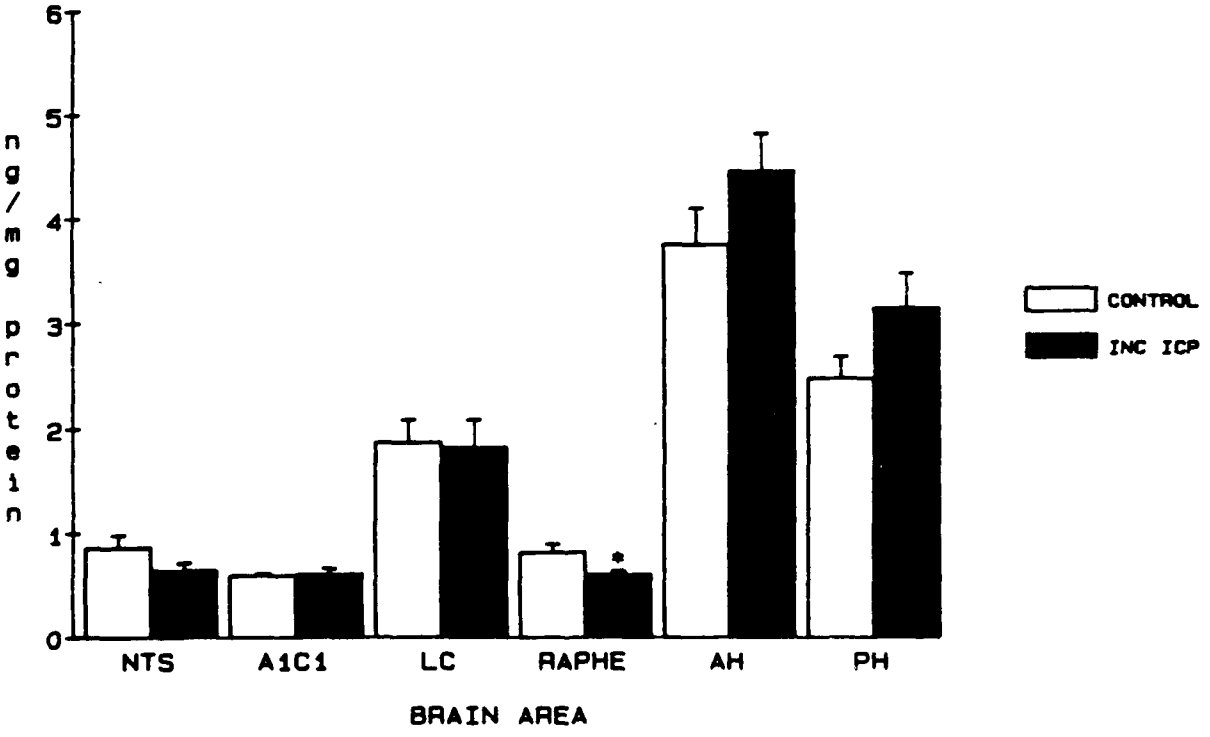
Fig. 7. EFFECTS OF INCREASED ICP ON 5-HIAA IN BRAINSTEM AREAS (NTS, A1C1, LC, RAPHE) AND THE HYPOTHALAMUS (AH, PH)



Results are Mean +/- SEM, (n=6), ** p<.01

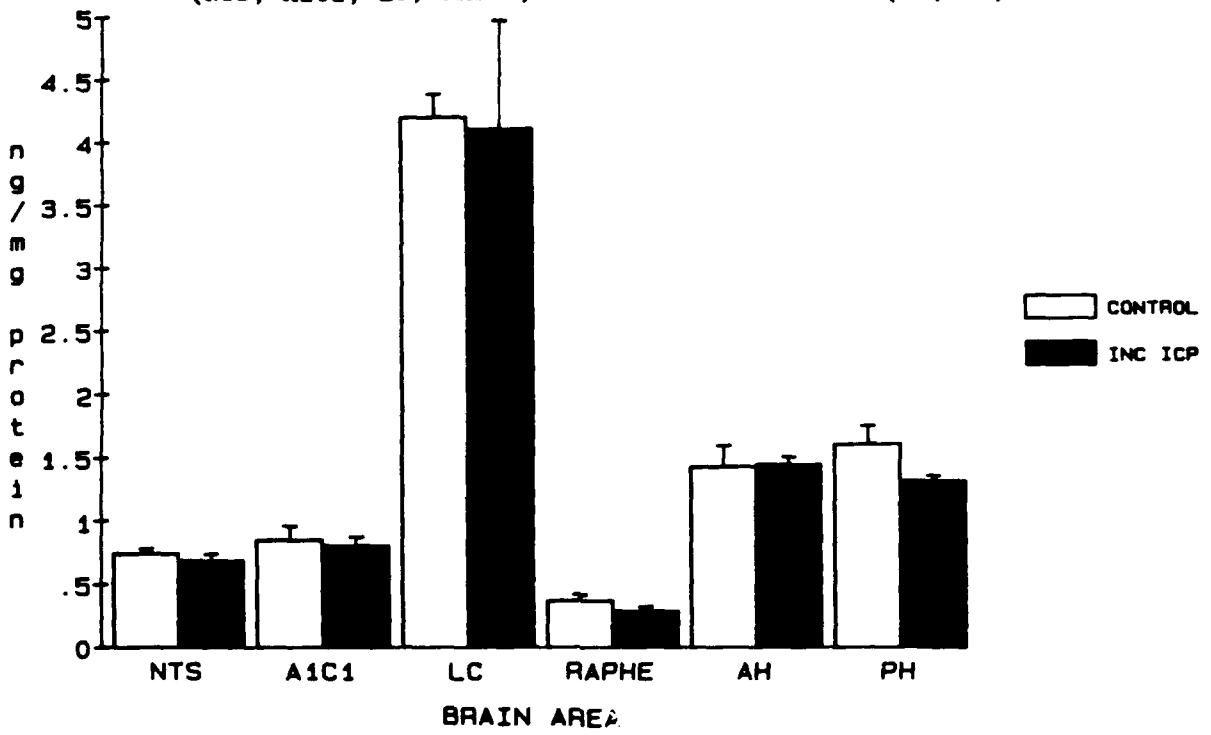
DA and its metabolite, HVA, were significantly decreased in the raphe nucleus by 27% and 32% respectively as a consequence of increased ICP (both $p<.05$). DOPAC, another metabolite of DA, was not affected in the raphe nuclei (Figs. 8-10; Table 7)

Fig. 8. EFFECTS OF INCREASED ICP ON DOPAMINE (DA) IN BRAINSTEM AREAS (NTS, A1C1, LC, RAPHE) AND THE HYPOTHALAMUS (AH, PH)



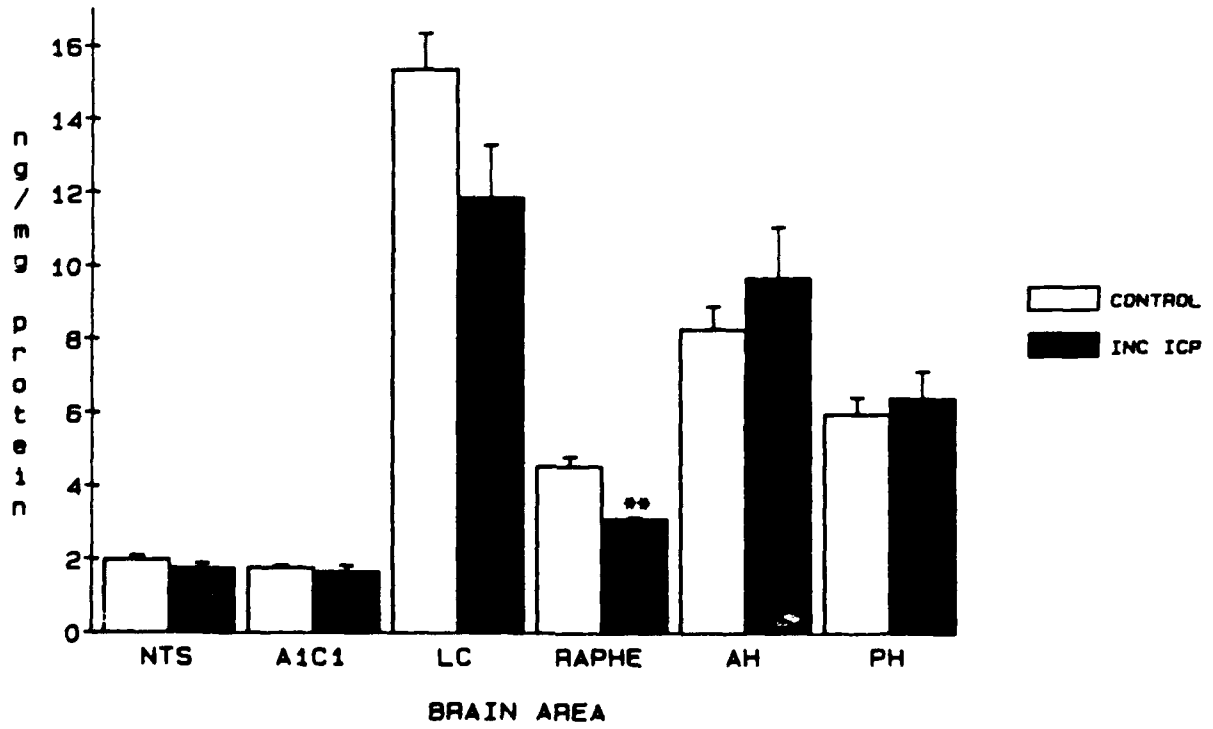
Results are Mean +/- SEM (n=6). * P<.05

Fig. 9. EFFECTS OF INCREASED ICP ON DOPAC IN BRAINSTEM AREAS (NTS, A1C1, LC, RAPHE) AND THE HYPOTHALAMUS (AH, PH)



Results are Mean +/- SEM (n=6).

Fig. 10. EFFECTS OF INCREASED ICP ON HVA IN BRAINSTEM AREAS (NTS, A1C1, LC, RAPHE) AND THE HYPOTHALAMUS (AH, PH)



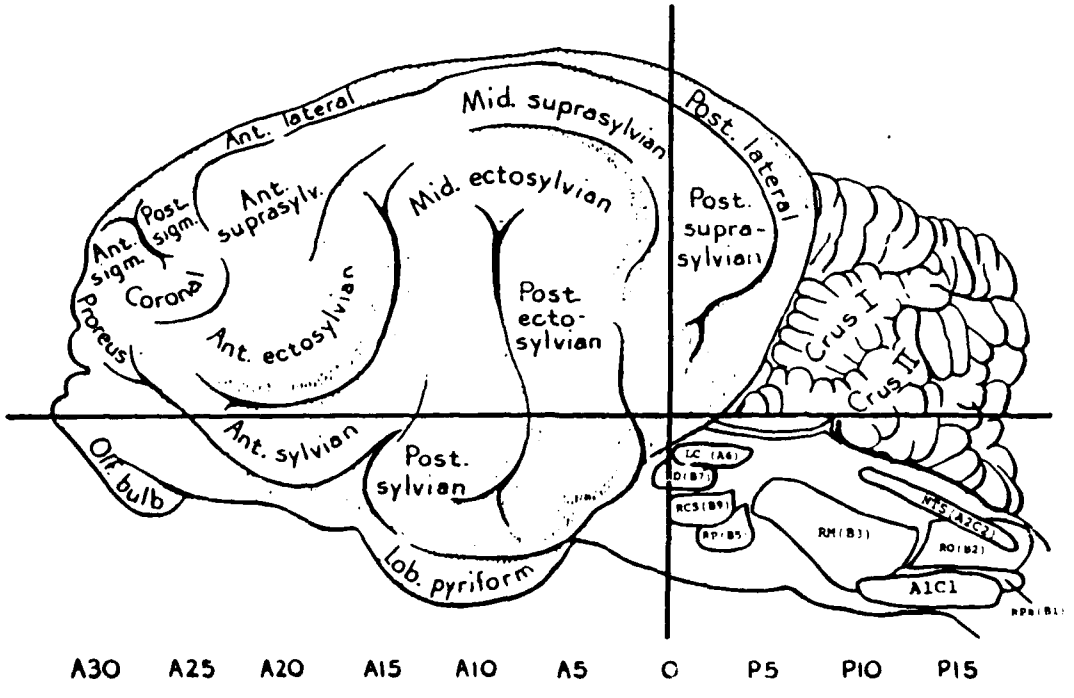
Results are Mean +/- SEM (n=6). ** p<.01

DISCUSSION

The Medulla: Biogenic Amine Changes In The Ventrolateral Medulla From Increased ICP

The ventrolateral area of the medulla (VLM) projects directly to the intermediolateral cell column of the spinal cord, the origin of the sympathetic preganglionic axons (31). It has been proposed, therefore, that the VLM contains tonic vasomotor neurons which help maintain MABP (2). Besides projecting caudally, the VLM also projects to the posterior hypothalamus (106). The VLM lies adjacent to the chemically sensitive areas of the ventral medullary surface (19,51,52) and contains neurons sensitive to CO_2 and H^+. Evidence exists that the VLM mediates the cerebral ischemic response and partakes in baroreceptor and peripheral chemoreceptor reflexes as well as some somatosympathetic reflexes. The VLM also influences vasopressin release (see review in 23).

The VLM appears to be divided into two parts: 1) The rostral vasopressor area (RVLM) containing epinephrine (EPI) neurons (area C1) and 2) a caudal vasodepressor portion (CVLM) which contains norepinephrine (NE) neurons (area A1) (16,101). The exact function of EPI in the RVLM is controversial; it may act as a vasopressor (104), vasodepressor (54,112) or either depending upon the type of adrenergic receptor with which it interacts (78).



Some investigators have felt that the RVLM (C1) EPI neurons exert a tonic excitatory action on the sympathetic preganglionic neurons of the intermediolateral cell column and thus, maintain the resting level of sympathetic tone in the cardiovascular effectors (50,104). Other investigators feel that the RVLM (C1) EPI neurons play a sympathoinhibitory role and act as vasodepressors (54,112). The EPI (C1) neurons of the RVLM themselves are thought to be under the tonic inhibitory control of GABAergic input from either the NTS or GABAergic neurons within the VLM itself (77). Neuropeptide Y and substance P are thought to co-transmitters in the EPI (C1) RVLM neurons and may be the primary neuroeffectors from the EPI containing neurons (15,36,41,72). Other substances such as glutamic acid, GABA, glycine, acetylcholine and clonidine which also affect VLM function will not be considered (9,16,17,31,46,52,80,98,103,104,132,133,135).

The VLM area sampled in our experiments represents a mixture of the caudal A1 (NE-depressor) area and the rostral C1 (EPI-pressor) area. Hence, our VLM sample is referred to as area A1C1. Following ICP elevations sufficient to induce a pressor response, in the A1C1 area there were large significant decreases in EPI (68%) as well as small decreases in NE (14%) and 5-HIAA (14%). Even though the A1 and C1 areas were not distinguished separately by our technique the decrease in EPI was many times that of NE. If EPI in the RVLM is indeed crucial in maintaining sympathetic tone it would appear that the decreased EPI levels would be either a response to the ICP-induced MABP rise (attempting to return MABP to normal) or a response to the ICP itself. If the RVLM C1 EPI neurons acts as a vasodepressor, the decrease in A1C1 EPI levels which occurred followed ICP elevation may have been a requisite in order to allow or facilitate the MABP rise.

Other investigators have performed In-vivo voltammetric measurements and have demonstrated demonstrated that increasing MABP by I.V. phenylephrine infusion reduced catecholamine release in both the RVLM and CVLM, while decreasing MABP by I.V. nitroprusside increased RVLM and CVLM catecholamine release (14,99). Unfortunately, in-vivo voltammetry cannot distinguish catecholamine components so the relative changes of EPI and NE with increased or decreased ICP are unknown.

The A1C1 area also contains NE and microdialysis techniques have demonstrated decreased A1C1 NE release consequent to increased MABP induced by intraventricular hypertonic saline administration (61). Furthermore, selective administration of NE to area A1C1 by microdialysis alleviated the pressor response consequent to intraventricular saline (61). These results support the hypothesis that A1C1 NE neurons inhibits sympathetic tonus either directly or indirectly (16,49). Increasing ICP evoked no significant A1C1 NE changes in our experiments so the A1C1 EPI decreases appears

the more important A1C1 biogenic amine change in the Cushing pressor response. But whether the A1C1 EPI changes enhances or inhibits sympathetic output cannot be ascertained from our experiments.

Since increased ICP evokes an increase in sympathetic nerve activity (SNA) and MABP (20,39,55,64,84,111,127), it is very similar to a stress response. Footshock stress has been shown to decrease EPI and NE levels in the rostral C1 but not the caudal C1 area. Moreover, after phenylethanolamine-N-mrthyltransferase (PNMT) inhibition, EPI and NE levels were decreased in both the rostral and caudal C1 areas. The authors concluded that footshock stress decreased the steady state level and increased the turnover of EPI (113). Immobilization stress significantly decreased EPI levels and reduced (nonsignificant) NE in the A1(C1) area (109) and increased the turnover of NE, but not DA or 5-HT (27). Stress induced by hindlimb ischemia in anesthesized rats resulted in decreased NE and increased 5-HIAA levels in the brain stem (120) and also increased the level of the NE metabolite, MHPG-SO_4, which is indicative of increased NE turnover and utilization, in the rostral and caudal brain stem (121).

Thus, the decreased levels of EPI we found in area A1C1 are probably related to cardiovascular effects from increased SNA as a consequence of stress. The data are not sufficient to determine if the depletions were because of increased utilization or decreased neuronal activity. Whether this change reflects a causative factor in the increase in MABP (i.e. vasopressor) or a compensatory role (i.e. vasodepressor) also cannot be determined at this time.

The Medulla: Biogenic Amine Changes In The Nucleus Tractus Solitarius (NTS) From Increased ICP

Baroreceptors are stretch receptors in the wall of the heart and blood vessels. Baroreceptor afferent fibers from the carotid sinus and aortic arch run in the ninth and tenth cranial nerve and terminate in the NTS (22,69). NTS neurons give off axons collaterals to the dorsal motor nucleus of the vagus nerve and also synapse with the VLM (8) which, as we have said, strongly influences sympathetic tone throughout the arterial tree. Thus, stretch receptors in the major great vessels and heart sense MABP and heart rate and provide this information to the brain stem vagal centers which may decrease the cardiac rate and to the brain stem centers which may increase heart rate and alter vasomotor tone. Hence, heart rate and vascular tone are reflexively controlled through medullary centers.

Electrical stimulation of the NTS lowers MABP (115) while bilateral ablation of this nucleus abolishes the baroreceptor reflex and leads to acute fulminating neurogenic hypertension.

This hypertension is believed to be mediated by alpha-adrenergic receptors because such hypertension may be inhibited by I.V. phentolamine, an alpha-adrenergic blocking drug (32). ICP elevation can markedly enhance sympathetic nerve activity with a resulting loss of baroreceptor sensitivity (64,70), This tends to augment MABP and maintain CPP (75).

NE and EPI are implicated in NTS function but conflicting hypotheses have arisen whether NTS EPI and NE act as vasopressors or vasodepressors (42,44,63,88,128,136). The NTS also contains cell bodies for serotonin (5-HT) (73) but studies of this biogenic amine have provided conflicting results. The local application of 5-HT onto the NTS have demonstrated both a dose dependent increase in MABP in rats (134) and cats (25) as well as dose dependent decreases in MABP in rats (68).

Microinjection experiments indicated that GABA placed into the NTS increases MABP, causes tachycardia (18) and inhibit the baroreflex (129), while glutamate causes dose dependent decreases in MABP and heart rate (126).

In our experiments wherein we elevated ICP and caused a Cushing pressor response we found a significant 57% depletion of EPI in the NTS as well as smaller non-significant reductions of NE (18%) and DA (26%) (Figs. 4,5,8; Table 4).

In the cat, push-pull cannulae placed within the NTS revealed a significant decrease in EPI and NE release when MABP was increased by 47 mm/Hg by I.V. NE injection or by blood infusion. When MABP was reduced by bleeding, or I.V. injection of nitroprusside or chlorisondamine, no changes occurred in NTS EPI or NE release, but a slight reduction in DA release was seen (63). In-vivo voltammetry studies of biogenic amines within the NTS provided somewhat different results: increases in MABP by phenylephrine infusion decreased NE and increased 5-HIAA release and tissue levels. Decreases in MABP by nitoprusside infusion also decreased NE but had no effect on 5-HIAA release and tissue levels. By this technique any alteration in MABP appeared to lower the release and tissue levels of NE (12).

Stress provided by footshock decreased EPI and NE levels in the rostral C2 area (NTS) and was taken as evidence of increased EPI and NE turnover (113). Immobilization stress decreased EPI tissue levels in the caudal and medial NTS and decreased EPI and NE levels in the anterior NTS (109). Increased PNMT activity was also increased in the medial and anterior NTS (110) indicating enhanced EPI utilization was correlated with decreased EPI tissue levels.

Thus, our finding of significant depletions of EPI and decreases in NE is consistent with prior reports examining the effect of MABP alterations and/or stress on biogenic amines in

the NTS. We cannot, however, be certain if the EPI depletions were caused by increased utilization of EPI or decreased neuronal activity. The role of EPI in the NTS is not clear. Kobilansky et al. (63) suggested that NTS NE and EPI may act to increase MABP (vasopressor) since release was decreased when MABP was increased. The vasopressor function of NE within the NTS has much additional support (63,128,136). Others have suggested a vasodepressor role of EPI within the NTS based on results with spontaneously hypertensive rats (42,44,136).

The Locus Coeruleus

The locus coeruleus (LC) is the brain stem nucleus containing the largest number of noradrenergic (NE) neurons (30). The LC (A6 noradrenergic cell group) gives rise to the dorsolateral noradrenergic pathway (30), which projects rostrally to the frontal cortex, hippocampus (3,43), amygaloid complex (59), thalamus (59,62) and several hypothalamic areas including the supraoptic nucleus (40,59). NE nerve endings in the supraoptic nucleus terminate preferentially in those areas containing vasopressin (76), a peptide possessing strong pressor activity. The LC also sends descending projections to the dorsal raphe nucleus (40) and a number of primary cardiovascular areas including the VLM, dorsal vagal complex, nucleus ambiguus and the NTS (5,21,107,130,131).

The Locus Coeruleus and EPI and NE

Electrical stimulation of the LC enhances the release of NE and EPI in the posterior hypothalamus and is associated with an increased MABP. This systemic pressor response appears dependent on the posterior hypothalamus because lesions of the posterior hypothalamus or application of clonidine, a presynaptic alpha-adrenergic agonist, to the posterior hypothalamus attenuate the rise in MABP from LC stimulation (74,96). The vasopressor response to LC stimulation seems to be dependent upon descending hypothalamoadrenal pathways because LC stimulation is ineffective in adrenalectomized animals (53). But hypothalamic input per se may not be required for LC stimulation of the adrenal medulla because this effect remains intact following midcollicular transection (47).

Experimentally, increases in MABP or blood volume depress LC NE neuronal activity while decreases in MABP or blood volume enhance this activity (34,35,79,124).

Increased blood pressure in the carotid sinus also inhibits the activity of the supraoptic vasopressin neurons and this effect is abolished when a neurotoxin is injection into the LC (6).

These findings suggest a pressor role for LC NE neurons in the regulation of MABP: NE LC cells may act in parallel with

the sympathetic nervous system as the cardiovascular system is altered (79).

As may be expected in this confusing field, other studies have suggested a depressor role for LC NE (82,123).

A moderately dense network of EPI containing neurons has also been observed around the LC (57) and these cells supposedly inhibit LC function (4,102).

The Locus Coeruleus and Serotonin (5-HT)

Serotonin neurons have been shown to be present in the LC immunocytochemically (93). They influence influence NE metabolism of the cells within the LC (97).

In the LC we detected a significant depletion of EPI (66%) and a non-significant reduction of NE (26%). Serotonin functioning did not appear to be affected (Figs. 4-7; Table 6).

Others using in-vivo voltammetry and drug-induced MABP changes have observed increases in LC 5-HIAA release following either increases or decreases of MABP (13). In the same study (13) the effects of of drug-induced MABP increases on LC catecholamines was less clear but seemed to show an initial decrease in release in either NE or DA followed by an increased release. But drug-induced decreases in MABP did not affect LC catecholamines. Concomitant tissue analyses showed variable results and suggested that the measured catecholamine effects may have been due to DA (13). Other results indicate that experimentally-induced increases in MABP depress the activity of LC noradrenergic (NE) neurons while decreases in MABP enhance this activity (34,83,124). That is, activation of the LC will increase MABP while depression of the LC will decrease MABP.

Stress from footshock increased EPI turnover and decreased EPI and NE levels in the LC of rats (113). Immobilization stress decreased the levels of EPI, but not NE (109), and increased PNMT activity (110) in rats. Immobilization stress also increased the release of NE, as shown by in-vivo voltammetric analysis (48), and increased NE turnover (27).

The results of our experiments, whereby we artificially increased ICP, generally agree with those NE and EPI (especially) changes in the LC found in stress experiments. The decrease in EPI and moderate decrease in NE may be related to the simultaneous enhancement of sympathetic and LC activity. EPI has been hypothesized to be inhibitory to LC neurons (4,102). Whether the decreased levels reflect increased utilization or decreased neuronal activity cannot be determined from the data. The failure to find 5-HT or 5-HIAA changes

after ICP and MABP alterations may reflect the nature of the stress applied to these animals.

The Raphe Nuclei

Brain stem raphe nuclei also participate in cardiovascular control. The raphe nuclei consists of nine cell groups which contain serotonin (5-HT): B1= N. raphe pallidus, B2= N. raphe obscurus, B3=N. raphe magnus, B4= a group of cells below the fourth ventricle, B5=N. raphe pontis, B6= a group of cells in the midline below the fourth ventricle, B7= N. raphe dorsalis, B8= N. raphe medianus, B9= N. raphe centralis superior. Ascending fibers from the mesencephalic and rostral pontine nuclei, especially B7-B9, innervate the suprachiasmatic nucleus as well as hypothalamic, limbic and cortical areas (30). The B1-B3 cell group send descending serotonergic neurons to the intermediolateral cell column of the spinal cord (30,71). The raphe nuclei also project to the LC (24).

In anesthesized cats injections of 5-HT in low doses into the ventricular system increased MABP while higher doses decreased MABP. Subsequent studies in rats indicated that the 5-HT pressor response may have been mediated via the anterior hypothalamus while the depressor effect occurred because of serotonin's action on brain stem sites (25,117). Injection of 5-HTP, the precursor of 5-HT, into the feline fourth ventricle depresses MABP (125).

Pressor/Depressor Responses Of Various Raphe Nuclei

In cats, stimulation studies of the raphe nuclei revealed both pressor and depressor responses. Stimulation of B1 yielded predominantly pressor but also depressor responses; stimulation of the the anterior B2 increased MABP while stimulation of the posterior B2 decreased it. B3 also had an anterior and posterior depressor area (1). B7 stimulation causes an increase in MABP and heart rate and these effects are blocked by the 5-HT antagonist, methysergide (114).

In rats, stimulation of B7 and B8 also yields a pressor response (66,118). If, in rats, MABP were experimentally increased, B7 exhibited increased release of 5-hydroxyindoleacetic acid (5-HIAA) and decreased NE. Decreased MABP did not alter the release of either 5-HIAA or NE (33). Because increasing MABP was associated with an increase release of 5-HIAA while decreasing MABP did not affect 5-HIAA release, it was inferred that the B7 5-HIAA (5-HT) release was in response to the MABP rise and not causative of it. It was surmised that B7 was exhibiting a depressor function, the 5-HT release serving to try and return MABP to baseline (33,38). These results evaluating 5-HT/5-HIAA release are at variance with the some results of direct 5-HT application electrical stimulation of the raphe nuclei. Furthermore, other reports on

serotonergic bulbospinal neurons in the regulation of MABP have assigned the same nuclei both pressor and depressor roles (26,45,58,81,94). There are also species differences to pharmacological treatments in rats, cats and dogs (65).

Noradrenergic Innervation Of Raphe Nuclei

Although 5-HT predominates in the raphe nuclei, these nuclei also receive extensive noradrenergic innervation which may influence the firing rate of 5-HT neurons and inhibit 5-HT synthesis (7,100,114). Adrenergic nerve endings have been localized in the B1-B3, B7 and B9 cell groups (57) but their function is unknown.

We chose to examine the raphe complex as a whole. Our pooled raphe nuclei sample contained two rostral (B9 and B7) and two caudal (B1 and B3) nuclei. We found significant depletions of 5-HT (30%), 5-HIAA (36%), DA (27%), HVA (32%) and EPI (47%) (Figs. 4,6-8,10, Table 7) following increased ICP and the concomitantly occurring pressor response. The finding of decrease in 5-HT and DA as well as their metabolites, 5-HIAA and HVA respectively, suggests that general raphe functioning as well as dopaminergic input to the raphe were both depressed. The depletion of EPI is similar to that seen in the other brain stem areas (A1C1, NTS and LC) which we examined, but the meaning of the EPI depletion is unclear as there have been no other studies examining its function within the raphe system. Our general results cannot be used to speculate specifically on MABP effects, but probably do reflect the general effects of increased ICP.

The Hypothalamus

The hypothalamus receives fibers from most brain areas, including the limbic system and brain stem, as well as from peripheral receptor organs. It has been referred to by Sherrington as "the head ganglion of the autonomic system" because it receives, integrates and affects both parasympathetic and sympathetic functions. The hypothalamus also is intimately involved in neuroendocrine functions by virtue of its control over the pituitary gland.

Electrical stimulation of the posterior hypothalamus (PH) elicits a pressor response (60) which may be caused by the release of NE and EPI from catecholaminergic nerve terminals within the PH (95). Microinjections of EPI or NE into the PH increase MABP and heart rate (122) which appears to be mediated primarily by beta-adrenergic receptors (88). Perfusion of the PH with the dopamine will enhance the increase in MABP elicited by PH stimulation (89).

NE applied to the paraventricular N. of the PH enhances the release of vasopressin which, in turn, also induces a pressor response (10).

The PH is demonstrably under medullary control: electrical stimulation of the RVLM C1 area elicits a pressor response and increases the release of EPI in the PH (106).

Conversely, stimulation of the anterior hypothalamus (AH) by a a current of specific frequency and voltage decreases MABP and the depressor response appears to be mediated by alpha-adrenergic receptors (92). Injections of NE or EPI into the AH also lowers MABP and heart rate (122).

It appears, therefore, that catecholamines, especially NE and EPI, exert opposite cardiovascular effects in the two hypothalamic regions. Catecholamine release in the PH increase MABP and heart rate, while release in the AH decrease MABP and heart rate. Besides the catecholamines other hypothalamic neurotransmitters also appear to be involved in the hypothalamic control of MABP; these include GABA (91), Ach (87), vasopressin (37) and opioids (85,86).

In our experiments we found no significant biogenic amine changes in the AH. In the PH, however, significant depletions of NE (46%) and EPI (66%) occurred (Figs. 4,5; Tables 8,9).

In cats, elevation of MABP from splanchnic nerve stimulation increased the release of NE, EPI and DA in the AH. These substances were not elevated in the PH (90), Presumably AH elevations of these biogenic amines were to counteract the increase in MABP. Conversely, decreasing MABP by nitroprusside infusion or bleeding led to an increase in the release of NE, EPI and DA in the PH, but not in the AH (116). As catecholamine release in the PH tends to elevate MABP the increased release of these substances was compensatory for the fall in MABP. In both experiments it was shown, by brain stem transection, that the effects were probably mediated by catechloaminergic cell bodies in the pons/medulla. This was corroborated by experiments showing that electrical stimulation of the RVLM C1 area leads to a pressor response and increased EPI release in the PH (106).

Footshock and immobilization stress was shown to decrease EPI and NE in the whole hypothalamus of rats (105,113). Immobilization stress has been reported to increase the turnover and decrease the levels of NE in the whole hypothalamus (27) and decreased EPI levels in several hypothalamic nuclei (67). Stress caused by hindlimb ischemia in anesthesized rats decreased NE levels (120) and increased NE turnover as measured by MHPG-SO_4 (121).

Thus, it could be considered that the decreased levels of EPI and NE in the PH which we observed following ICP increases reflected a dramatic increase in SNA since increased ICP elicits a massive sympathetic discharge, even to the extent of overriding parasympathetic effects (64,75). We may speculate that enhanced PH monoamine utilization required by the necessary MABP increase to preserve CPP may have resulted in EPI and NE depletions if the syntheses rates could not keep pace with catecholamine utilization.

SUMMARY

The monoaminergic neurotransmitters (EPI, NE, 5-HT) and some their metabolites (DOPAC, HVA, 5-HIAA) were evaluated in selected brain stem areas and the hypothalamus after increases in ICP sufficient to elicit an immediate Cushing response.

Overall, in the brain stem areas (LC, area A1C1, NTS and Raphe) large depletions of EPI (47%-68%) occurred in every area (Fig. 4; Tables 4-7). Moderate, although not statistically significant, reductions (12%-26%) in NE were seen in every area (Fig. 5; Tables 4-7). The Raphe nuclei showed indications of reduced serotonergic (5-HT) and dopaminergic (DA) functioning. No remarkable effects occurred in the AH, but in the PH both EPI and NE were depleted 66% and 46% respectively (Figs. 4,5; Tables 8,9). We could not determine if the depletions or reductions were due to increased utilization or decreased functioning because our analytical conditions did not permit us to determine the metabolite of NE, MHPG, nor did we assay the EPI synthesis enzyme, PNMT.

The neurochemical changes consequent to the elevation of ICP and its concomitant Cushing pressor response closely mirror the alterations found in stress studies, especially in regards to EPI depletions (e.g. 109). Some similarity to stress would be hypothesized because increased ICP causes a massive increase in SNA leading to a cascade of subsequent sympathetic effects, such as increased plasma catecholamines and increases in MABP.

The results of this study, as with all other studies to date, did not determine if the neurochemical alterations are related to causative factors or reflect compensatory mechanisms secondary to the rise in MABP. The interpretation of our findings is open to speculation and must await further studies.

GENERAL SIGNIFICANCE

The effects of increased ICP on brain stem monoamines had not been previously evaluated and it was unknown whether increased ICP and accompanying brain stem compression led to any overall or generalized effects on brain stem monoaminergic functioning. Overall effects, such as depression or depletion of all monoamine systems, may suggest that brain stem functions

became severely disrupted indicating possible cellular dysfunction or death. Our results suggest that there were not global effects on the monoaminergic systems because there were only selective alterations: EPI overall and 5-HT and DA in the Raphe nuclei. The absence of global effects may suggest that the brain stem, as a whole, was not severely affected by short term (6 min) high pressure (120-140 mm Hg). Of course, this does not rule out global effects on other neurotransmitter systems (e.g. amino acids, acetylcholine) which may play a part in the brain stem response to increased ICP.

The present results will serve as a comparison for future brain injury results because rapid increases in ICP and immediate elicitation of the Cushing pressor response is also seen after brain wounding. This comparison will also us to ascertain if the effects due to brain wounding are a consequence of increased ICP or if there are other factors involved.

Table 1. EFFECT OF INCREASED INTRACRANIAL PRESSURE (ICP) ON MEAN ARTERIAL BLOOD PRESSURE (MABP)

	CONT	1 Min	2 Min	3 Min	4 Min	5 Min	6 Min
CONTROLS							
L1	108	106	97	106	100	100	100
L4	143	143	143	143	143	143	143
L9	125	132	123	123	127	127	133
L10	122	122	122	122	127	127	127
L11	102	102	102	102	102	102	103
L14	116	116	116	116	116	116	116
MEAN	119	120	117	119	119	119	120
+/-SEM	6	6	7	6	7	7	7
INC. ICP							
L8	131	220	210	208	226	242	242
L12	128	175	170	190	182	180	177
L13	150	190	223	230	230	225	213
L15	136	177	171	190	180	175	178
L16	122	202	180	177	217	220	203
L17	120	213	215	183	173	173	173
MEAN	128	182	196	196	201	203	198
+/-SEM	5	6	9	8	10	12	1

Values are in mm-Hg.

Table 2. EFFECT OF INCREASED INTRACRANIAL PRESSURE (ICP) ON CEREBRAL PERFUSION PRESSURE (CPP)

	CONT	1 Min	2 Min	3 Min	4 min	5 Min	6 Min
CONTROL							
L1	90	88	79	88	82	82	82
L4	135	135	135	135	135	135	135
L9	120	127	120	120	124	124	130
L10	117	117	117	117	122	122	122
L11	94	94	94	94	94	94	95
L14	104	104	104	104	104	104	104
MEAN	110	111	108	110	110	110	111
+/-SEM	7	8	8	7	8	8	9
INC. ICP							
L8	116	80	70	68	87	102	102
L12	113	35	80	100	55	70	81
L13	136	50	84	90	145	140	128
L15	105	47	47	60	61	40	58
L16	110	62	72	69	77	80	63
L17	108	73	75	62	48	48	48
MEAN	115	59	71	75	79	80	80
+/-SEM	5	7	5	7	14	15	12

Values are in mm-Hg.

Table 3. INTRACRANIAL PRESSURE (ICP) OF CATS USED IN INCREASED ICP EXPERIMENTS

	CONT	1 Min	2 Min	3 Min	4 Min	5 Min	6 Min
CONTROLS							
L1	18	18	18	18	18	18	18
L4	8	8	8	8	8	8	8
L9	5	5	3	3	3	3	3
L10	5	5	5	5	5	5	5
L11	8	8	8	8	8	8	8
L14	12	12	12	12	12	12	12
MEAN	9	9	9	9	9	9	9
+/-SEM	2	2	2	2	2	2	2
INC. ICP							
L8	16	140	140	140	139	140	140
L12	15	140	90	90	127	110	96
L13	14	140	135	140	85	85	85
L15	10	130	130	130	119	135	120
L16	12	140	108	108	140	140	140
L17	12	140	140	121	125	125	125
MEAN	13	137	124	122	123	125	120
+/-SEM	1	2	8	8	17	9	10

Values are in mm-Hg.

Table 4. EFFECT OF INCREASED ICP ON BIOGENIC AMINES AND METABOLITES IN THE NUCLEUS TRACTUS SOLITARIUS (NTS)

	NE	EPI	DA	DOPAC	HVA	5-HT	5-HIAA
CONTROLS							
L1	11.897	0.843	0.780	0.491	1.723	20.832	15.885
L4	19.208	1.290	1.338	0.801	2.369	21.479	7.990
L9	11.826	1.194	0.689	0.671	1.666	17.554	9.406
L10	10.704	0.698	0.533	0.630	2.183	24.831	13.995
L11	10.949	0.957	0.781	0.825	1.820	20.996	18.092
L14	12.976	0.908	1.060	1.037	2.152	24.043	10.273
MEAN	12.927	0.982	0.864	0.743	1.986	21.623	12.607
+/-SEM	1.298	0.049	0.118	0.036	0.117	1.061	1.631
INC. ICP							
L8	6.106	0.458	0.432	0.572	1.866	19.302	13.140
L12	13.058	0.499	0.750	0.557	1.431	20.615	9.242
L13	14.411	0.476	0.840	0.789	2.280	21.104	12.777
L15	11.390	0.479	0.479	0.867	1.471	15.092	5.444
L16	9.829	0.185	0.512	0.575	1.429	17.342	7.153
L17	13.696	0.425	0.826	0.729	2.011	21.691	10.249
MEAN	11.415	0.420*	0.640	0.682	1.748	19.191	9.668
+/-SEM	1.259	0.048	0.076	0.054	0.147	1.036	1.244

Values are pg/mg protein.
* $p < .01$

Table 5. EFFECT OF INCREASED ICP ON BIOGENIC AMINES AND METABOLITES IN THE AREA A1C1

	NE	EPI	DA	DOPAC	HVA	5-HT	5-HIAA
CONTROLS							
L1	12.679	0.849	0.674	0.595	1.612	17.875	14.530
L4	9.960	1.029	0.571	0.718	2.019	15.615	10.414
L9	10.044	0.801	0.589	0.559	1.686	17.561	9.433
L10	9.054	0.837	0.542	1.018	1.597	18.959	11.857
L11	6.912	0.816	0.594	0.995	1.777	15.604	20.341
L14	8.055	0.764	0.578	1.217	1.939	16.746	11.002
MEAN	9.451	0.849	0.591	0.850	1.772	17.060	12.930
+/-SEM	0.808	0.038	0.018	0.108	0.071	0.542	1.642
INC. ICP							
L8	5.847	0.376	0.715	0.587	1.651	19.434	11.346
L12	11.889	0.340	0.781	1.045	1.705	20.552	10.700
L13	9.989	0.344	0.605	0.941	2.375	15.133	13.192
L15	8.272	0.227	0.515	0.640	1.363	14.154	5.769
L16	5.895	0.246	0.393	0.787	1.206	15.553	7.825
L17	6.609	0.079	0.609	0.821	1.641	16.179	10.780
MEAN	8.084	0.269*	0.603	0.804	1.657	16.834	9.935
+/-SEM	1.002	0.045	0.057	0.071	0.164	1.044	1.091

Values are in pg/mg protein.
* p <.001

Table 6. EFFECT OF INCREASED ICP ON BIOGENIC AMINES AND METABOLITES IN THE LOCUS COERULEUS (LC)

	NE	EPI	DA	DOPAC	HVA	5-HT	5-HIAA
CONTROLS							
L1	28.071	0.959	2.469	4.071	13.165	25.933	28.959
L4	28.191	1.885	2.335	4.800	17.711	27.206	27.637
L9	21.920	0.903	1.582	3.930	15.889	25.231	19.236
L10	16.644	0.766	1.170	3.543	12.268	26.446	33.017
L11	18.316	0.963	1.474	4.204	14.718	22.439	38.017
L14	21.482	0.891	2.193	4.590	18.263	26.213	30.911
MEAN	22.437	1.061	1.871	4.190	15.336	25.578	29.630
+/-SEM	1.971	0.167	0.217	0.186	0.984	0.681	2.556
INC. ICP							
L8	6.131	0.453	0.591	1.112	10.862	21.908	33.426
L12	20.961	0.375	2.059	6.706	14.084	24.442	25.286
L13	21.746	0.442	2.520	6.334	17.628	23.709	31.619
L15	17.006	0.270	1.751	3.436	7.615	23.136	9.259
L16	17.923	0.416	1.981	3.047	10.301	23.048	15.622
L17	16.240	0.238	2.026	3.940	10.446	21.009	19.933
MEAN	16.668	0.366*	1.821	4.096	11.823	22.875	22.524
+/-SEM	2.288	0.037	0.267	0.862	1.434	0.506	3.828

Values are pg/mg protein.
* p <.01

Table 7. EFFECT OF INCREASED ICP ON BIOGENIC AMINES AND METABOLITES IN THE RAPHE NUCLEI

	NE	EPI	DA	DOPAC	HVA	5-HT	5-HIAA
CONTROLS							
L1	12.363	0.449	0.931	0.215	5.313	27.513	39.492
L4	10.142	0.724	0.657	0.484	5.110	22.440	31.858
L9	12.510	0.698	0.738	0.341	4.491	19.595	22.393
L10	14.472	1.970	1.139	0.530	4.554	30.158	37.901
L11	12.782	0.606	0.623	0.291	3.923	25.574	32.071
L14	16.775	0.913	0.807	0.363	3.822	18.491	31.934
MEAN	13.174	0.893	0.816	0.371	4.536	23.962	32.608
+/-SEM	0.915	0.224	0.079	0.048	0.246	1.870	2.457
INC. ICP							
L8	10.879	0.615	0.622	0.271	3.228	14.528	19.877
L12	11.864	0.360	0.555	0.253	2.902	14.904	18.664
L13	7.273	0.182	0.520	0.287	3.097	16.765	30.258
L15	8.254	0.258	0.477	0.459	2.962	14.457	11.007
L16	14.123	0.968	0.657	0.196	3.117	18.805	18.370
L17	12.207	0.464	0.754	0.258	3.213	20.740	26.100
MEAN	10.767	0.475*	0.598*	0.287	3.087**	16.700**	20.713**
+/-SEM	1.050	0.117	0.041	0.037	0.054	1.060	2.731

Values are pg/mg protein.
* p <.05, ** p <.01

Table 8. EFFECT OF INCREASED ICP ON BIOGENIC AMINES AND METABOLITES IN THE ANTERIOR HYPOTHALAMUS (AH)

	NE	EPI	DA	DOPAC	HVA	5-HT	5-HIAA
CONTROLS							
L1	45.180	3.159	4.617	0.864	6.600	24.259	14.732
L4	50.728	4.926	3.044	1.838	6.982	14.550	10.988
L9	35.211	2.665	2.911	1.256	10.197	16.047	11.749
L10	50.948	3.394	3.523	1.306	7.228	22.487	14.026
L11	37.087	2.477	3.181	1.356	9.000	19.264	18.463
L14	43.846	3.110	5.141	1.961	9.576	22.460	12.843
MEAN	43.833	3.289	3.736	1.430	8.264	19.845	13.800
+/-SEM	2.707	0.355	0.377	0.165	0.619	1.593	1.091
INC. ICP							
L8	30.917	1.773	6.012	1.716	12.638	21.553	14.606
L12	48.710	3.245	4.352	1.340	7.217	14.367	10.683
L13	36.432	2.398	4.480	1.361	9.775	21.028	16.551
L15	52.617	2.976	4.189	1.409	5.801	16.242	5.532
L16	37.215	3.131	3.283	1.320	14.748	18.633	11.606
L17	39.247	2.232	4.477	1.503	7.801	23.359	15.518
MEAN	40.856	2.626	4.465	1.442	9.663	19.197	12.416
+/-SEM	3.338	0.238	0.360	0.061	1.403	1.398	1.659

Values are ng/mg protein.

Table 9. EFFECT OF INCREASED ICP ON BIOGENIC AMINES AND METABOLITES IN THE POSTERIOR HYPOTHALAMUS (PH)

	NE	EPI	DA	DOPAC	HVA	5-HT	5-HIAA
CONTROLS							
L1	31.262	2.067	1.863	1.376	4.453	8.227	9.201
L4	54.142	3.335	3.317	2.238	6.355	9.745	7.731
L9	48.936	4.189	2.188	1.382	6.962	16.427	8.862
L10	31.312	2.082	2.560	1.800	4.881	12.907	9.154
L11	38.973	1.395	2.702	1.472	6.000	17.103	12.501
L14	39.919	3.254	2.279	1.388	7.160	15.045	9.315
MEAN	40.757	2.720	2.484	1.609	5.969	13.242	9.461
+/-SEM	3.783	0.425	0.205	0.142	0.449	1.481	0.653
INC. ICP							
L8	17.281	0.707	3.957	1.160	8.010	17.546	11.087
L12	21.534	0.806	4.104	1.324	6.090	16.929	9.387
L13	21.677	0.870	2.212	1.306	6.387	13.344	10.886
L15	25.795	1.668	3.028	1.294	3.904	12.182	3.549
L16	31.736	1.098	3.332	1.477	8.888	20.648	9.982
L17	14.484	0.384	2.286	1.306	5.065	11.822	9.100
MEAN	22.085*	0.922*	3.153	1.311	6.390	15.412	8.999
+/-SEM	2.504	0.177	0.328	0.041	0.750	1.436	1.137

Values are ng/mg protein.
* $p < .01$

REFERENCES

1 Adair, J.R., Hamilton, B.L., Scappaticci, K.A., Helke, C.J., and Gillis, R.A. Cardiovascular responses to electrical stimulation of the medullary raphe area of the cat. Brain Res. 128: 141-145, 1977.

2 Amendt, J., Czachurski, J., Dembowsky, K., and Seller, H. Bulbospinal projections to the intrmediolateral cell column: A neuroanatomical study. J. Auton. Nerv. Syst. 1: 103-117, 1979.

3 Anden, N.E., Dahlstrom, A., Fuxe, K., Larsson, K., Olson, L., Ungerstedt, and U. Ascending monoamine neurons to the telencephalon and diencephalon. Physiol. Scand. 67: 313-326, 1966.

4 Astier, B., Kitahama, K., Denoroy, L., Berod, A., Jouvet, M., and Renaud, B. Biochemical evidence for an interaction between adrenaline and noradrenaline neurons in the rat brainstem. Brain Res. 397: 333-340, 1986.

5 Aston-Jones, G., Ennis, M., Pierbone, V.A., Nickell, W.T., and Shipley, M.T. The brain locus coeruleus: restricted afferent control of a broad efferent network. Science 234: 734-737, 1986.

6 Banks, D. and Harris, M.C. Lesiona of the locus coeruleus abolish baroreceptor-induced depressions of supraoptic neurons in the rat. J. Physiol. (Lond.). 355: 383-398, 1984.

7 Baraban, J.M., Wang, R.Y., and Aghajanian, G.K. Reserpine suppression of dorsal raphe neuronal firing: Mediation by adrenergic system. Eur. J. Pharmacol. 52: 27-36, 1978.

8 Benarroch, E.E., Granata, A.R., Giuliano, R., and Reis, D.J. Neurons of the C1 area of the rostral venrolateral medulla mediate nucleus tractus solitarii hypertension. Hypertension (Suppl. 2) 8: 156-160, 1986.

9 Benarroch, E.E., Granata, A.R., Ruggerio, D.A., Park, D.H., and Reis, D.J. Neurons of the C1 area mediate cardiovascular responses initiated from the ventral medullary surface. Am. J. Physiol. 250: R932-R945, 1986.

10 Benetos, A., Gavras, I., and Gavras, H. Norepinephrine applied in the paraventricular hypothalamic nucleus stimulates vasopressin release. Brain Res. 381: 322-326, 1986.

11 Berman, A.L. The brainstem of the cat. A cytoarchitectonic atlas with coordinates. Madison, WI.: Univ. of Wisconsin Press. 1968.

12 Bhaskaran, D. and Freed, C.M. Changes in arterial blood pressure lead to baroreceptor-mediated changes in norepinephrine and 5-hydroxyindoleacetic acid in rat nucleus tractus solitarius. J. Pharmacol. Exp. Ther. 245: 356-363, 1988.

13 Bhaskaran, D. and Freed, C.R. Changes in neurotransmitter turnover in locus coeruleus produced by changes in arterial blood pressure. Brain Res. Bull. 21: 191-199, 1987.

14 Bhaskaran, D. and Freed, C.R. Catechol and indole metabolism in the rostral ventrolateral medulla change synchronously with changing blood pressure. J. Pharmacol. Exp. Ther. 249: 660-666, 1989.

15 Blessing, W.W., Oliver, J.R., Hodgson, A.H., Joh, T.H., and Willoughby, J.D. Neuropeptide Y-like immunoreactive C1 neurons in the rostral ventrolateral medulla of the rabbit project to sympathetic preganglionic neurons in the spinal cord. J. Auton. Nerv. Syst. 18: 121-129, 1987.

16 Blessing, W.W. and Reis, D.J. Inhibitory cardiovascular function of neurons in the caudal entrolateral medulla of the rabbit: Relationship to the a ea containing A1 noradrenergic cells. Brain Res. 253: 161-171, 1982.

17 Blessing, W.W. and Reis, D.J. Evidence that GABA and glycine-like inputs inhibit vasodepressor neurons in the caudal ventrolateral medulla of the rabbit. Neurosci. Lett. 37: 57-62, 1983.

18 Bousquet, P., Feldman, J., Bloch, R., and Schwartz, J. Evidence for a neuromodulatory role of GABA at the first synapse of the baroreceptor reflex pathway. Effect of GABA derivatives injected into the NTS. Naunyn-Schmiedeberg's Arch. Phamacol. 319: 168-171, 1982.

19 Bousquet, P., Feldman, J., Velly, J., and Bloch, R. Role of the ventral surface of the brain stem in the hypotensive effect of clonidine. Eur. J. Pharmacol. 34: 151-156, 1975.

20 Brown, F.K. Cardiovascular effects of acutely raised intracranial pressure. Am. J. Physiol. 185: 510-514, 1956.

21 Chu, N.S. and Bloom, F.E. The catecholamine-containing neurons in the cat dorsolateral pontine tegmentum: Distribution of the cell bodies and some axonal projections. Brain Res. 66: 1-21, 1974.

22 Ciriello, J. and Calaresu, F.R. Projection from buffer nerves to the nucleus of the solitary tract: An anatomical and physiological study in the cat. J. Auton. Nerv. Syst. 4: 299-310, 1981.

23 Ciriello, J., Caverson, M.M., and Polosa, C. Function of the ventrolateral medulla in the control of the circulation. Brain Res. Rev. 11: 359-391, 1986.

24 Conrad, L.C.A., Leonard, C.M., and Pfaff, D.W. Connections of the median and dorsal raphe nuclei in the rat: an autoradiographic and degenerative study. J. Comp. Neurol. 156: 179-206, 1974.

25 Coote, J.H., Dalton, D.W., Feniuk, W., and Himphery, P.P.A. The location of the sympatho-inhibitory action of 5-hydroxytrytamine given intracerebroventricularly. J. Physiol. (Lond.). 365: 29P, 1985.

26 Coote, J.H. and Macleod, V.H. The influence of bulbospinal monoaminergic pathways on sympathetic nerve activity. J. Physiol. (Lond.). 241: 453-475, 1974.

27 Corrodi, H., Fuxe, K., and Hokfelt, T. The effect of immobilization stress on the activity of central monoamine neurons. Life Sci. 7: 107-112, 1968.

28 Cushing, H. Concerning a definite regulatory mechanism of the vasomotor center which controls blood pressure during cerebral compression. Bull. J. Hopkins Univ. 12: 290-292, 1901.

29 Cushing, H. Some experimental and clinical observations concerning states of increased intracranial hypertension. Amer. J. Med. Sci. 124: 375-400, 1902.

30 Dahlstrom, A. and Fuxe, K. Evidence for the existence of monoamine containing neurons in the central nervous system. Acta Physiol. Scand. 62 (suppl. 232): 1-55, 1964.

31 Dampney, R.A.L. and Moon, E.A. Role of ventrolateral medulla in vasomotor response to cerebral ischemia. Am. J. Physiol. 239: H349-H358, 1980.

32 Doba, N. and Reis, D.J. Acute fulminating hypertension produced by brinstem lesions in the rat. Circ. Res. 32: 584-593, 1973.

33 Echizen, H. and Freed, C.R. Altered serotonin and norepinephrine metabolism in rat dorsal raphe nucleus after drug-induced hypertension. Life Sci. 34: 1581-1589, 1984.

34 Elam, M., Svensson, T.H., and Thoren, P. Differentiated cardiovascular afferent regulation of locus coeruleus neurons and sympathetic nerves. Brain Res. 358: 77-84, 1985.

35 Elam, M., Yao, T., Svensson, T.H., and Thoren, P. Regulation of locus coeruleus neurons and splanchnic sympathetic nerves by cardiac afferents. Brain Res. 290: 281-287, 1984.

36 Everitt, B.J., Hokfelt, T., Terenius, L., Tatemoto, K., Mutt, V., and Goldstein, M. Differential co-existence of neuropeptide Y (NPY)-like immunoreactivity with catecholamines in the central nervous system of the rat. Neuroscience 11: 443-462, 1984.

37 Feurstein, G., Zerbe, R.L., and Faden, A.I. Central cardiovascular effects of vasotocin, oxytocin and vasopressin in conscious rats. J. Pharmacol. Exp. Ther. 228: 348-353, 1984.

38 Freed, C.R., Echizen, H., and Bhaskaran, D. Brain serotonin and blood pressure regulation: studies using in vivo electrchemistry and direct tissue assay. Life Sci. 37: 1783-1793, 1985.

39 Freeman, N.E. and Jeffers, W.A. Effect of progressive sympathectomy on hypertension produced by increased intracranial pressure. Am. J. Physiol. 128: 662-671, 1940.

40 Fuxe, K. Evidence for the existence of monoamine neurons in the central nervous system. Acta Physiol. Scand. 64 (suppl. 247): 39-85, 1965.

41 Fuxe, K., Agnati, L.F., Harfstrand, A., Zini, I., Tatemoto, K., Pich, E.M., Hokfelt, T., Mutt, V., and Terenius, L. Central administration of neuropeptide Y induces hypotension bradypnea and EEG synchronization in the rat. Acta Physiol. Scand. 118: 189-192, 1983.

42 Fuxe, K., Ganten, D., Jonsson, G., Bolme, P., Agnati, L.F., Andersson, K., Goldstein, M., and Hokfelt, T. Evidence for a selective reduction of adrenaline turnover in the dorsal midline area of the caudal medulla of young spontaneously hypertensive rats. Acta Physiol. Scand. 107: 397-399, 1979.

43 Fuxe, K., Hamberger, B., and Hokfelt, T. Distribution of noradrenaline nerve terminals in the cortical areas of the rat. Brain Res. 8: 125-131, 1968.

44 Fuxe, K., Harfstrand, A., Agnati, L.F., Kalia, M., Neumeyer, A., Svensson, T.H., Von Euler, G., Terenius, L., Bernadi, P., and Goldstein, M. Physiological and biochemical characteristics of epinephrine/neuropeptide Y-costoring neurons in the medulla oblongata of the rat, with particular reference to vasodepressor functions within the nucleus tractus solitarii. In: Stolk, J.M., U'Prichard, D. and Fuxe, K., eds. Epinephrine in the central nervous system. New York: Oxford University Press. 1988, 346-365.

45 Gilbey, M.P., Coote, J.H., Macleod, V.H., and Peterson, D.F. Inhibition of sympathetic activity by stimulating in the raphe nuclei and the role of 5-hydroxytryptamine in this effect. Brain Res. 226: 131-142, 1981.

46 Giuliano, R., Ruggerio, D.A., Morrison, S., Ernsberger, P., and Reis, D.J. Cholinergic regulation of arterial pressure by the C1 area of the rostral ventrolateral medulla. J. Neuroscience 9(3): 923-942, 1989.

47 Goadsby, P.J. Brainstem activation of the adrenal medulla in the cat. Brain Res. 327: 241-248, 1985.

48 Gonon, F., Buda, M., De Simoni, G., and Pujol, J-F. Catecholamine metabolism in the rat locus coeruleus as studied by in vivo differential pulse voltammetry. II. Pharmacological and behavioral study. Brain Res. 273: 207-216, 1983.

49 Granata, A.R., Numao, Y., Kumada, M., and Reis, D.J. A1 noradrenergic neurons tonically inhibit sympathoexcitatory neurons of C1 area in rat brainstem. Brain Res. 377: 127-146, 1986.

50 Granata, A.R., Ruggiero, D.A., Park, D.H., Joh, T.H., and Reis, D.J. Brain stem area with C1 epinephrine neurons mediates baroreflex vasodepressor functions. Am. J. Physiol. (Heart 17) 248: H547-H567, 1985.

51 Guertzenstein, P.G. Blood pressure effects obtained by drugs applied to the ventral surface of the brain stem. J. Physiol. (Lond.). 229: L395-408, 1973.

52 Guertzenstein, P.G. and Silver, A. Fall in blood pressure produced from discrete lesions of the ventral surface of the medulla by glycine and lesions. J. Physiol. (Lond.). 242: 489-503, 1974.

53 Gurtu, S., Pant, K.K., Sinha, J.N., and Bhargava, K.P. An investigation into the mechanism of cardiovascular responses elicited by electrical stimulation of locus coeruleus and subcoeruleus in the cat. Brain Res. 301: 59-64, 1984.

54 Guyenet, P.G. and Stornetta, R.L. Inhibition of sympathetic preganglionic discharges by epinephrine and alpha-methylepinephrine. Brain Res. 235: 271-283, 1982.

55 Hoff, J.T. and Mitchell, R.A. The effect of hypoxia on the Cushing Response. In: Brock, M. and Dietz, H. eds. Intracranial Pressure. New York: Springer-Verlag. 1972, 205-209.

56 Hoff, J.T. and Reis, D.J. Localization of regions mediating mediating the Cushing response in CNS of the cat. Arch. Neurol. 23: 229-240, 1970.

57 Hokfelt, T., Johansson, O., and Goldstein, M. Central catecholamine neurons as revealed by immunohistochemistry with special reference to adrenaline neurons. In: Bjorklund, A. and Hokfelt, T., eds. Handbook of neuroanatomy. Vol. 2.: Classical neurotransmitters in the CNS, Part 1. New York: Elsevier Press. 1984, 157-276.

58 Howe, P.R.C., Kuhn, D.M., Minson, J.B., Stead, B.H., and Chalmers, J.P. Evidence for a bulbospinal serotonergic pressor pathway in the rat brain. Brain Res. 270: 29-36, 1983.

59 Jones, B.E. and Moore, R.Y. Ascending projections of the locus coreuleus in the rat. II. Autoradiographic study. Brain Res. 127: 23-53, 1977.

60 Karplus, J.P. and Kreidl, A. Gehirn und Sympathicus. VII. Mitteilung. Uber Beziehungen der Hypothalamuszentren zu Blutdruk und innerer Sekretion. Pflugers Arch. 215: 667-670, 1927.

61 Katahira, K., Mikami, H., Tsunetoshi, T., Kohara, K., Otsuka, A., Nagano, M., and Ogihara, T. Increase in the blood pressure and decrease in the norepinephrine release in the ventrolateral medulla during intraventricular administration of hypertonic NaCl. Pflugers Arch. Eur. J. Physiol. 414: 719-725, 1989.

62 Kobayashi, R.M., Palkovits, M., Kopin, I.J., and Jacobowitz, D.M. Biochemical mapping of noradrenergic nerves arising from the rat locus coeruleus. Brain Res. 77: 269-279, 1974.

63 Kobilansky, C., Lanzinger, I., and Philippu, A. Release of endogenous catecholamines in the nucleus tractus solitarii during experimentally induced blood pressure changes. Naunyn-Schmiedeberg's Arch. Pharmacol. 337: 125-130, 1988.

64 Kocsis, B., Fedina, L., and Pasztor, E. Two phase change of sympathetic rhythms in brain ischemia, Cushing reaction, and asphyxia. Am. J. Physiol. 256 (Regulatory Integrative Comp. Physiol. 25): R120-R132, 1989.

65 Kuhn, D.M., Wolf, W.A., and Lovenberg, W. Review of the role of central serotonergic neuronal system in blood pressure regulation. Hypertension 2: 243-255, 1980.

66 Kuhn, D.M., Wolf, W.A., and Lovenberg, W. Pressor effects of electrical stimulation of the dorsal and median raphe nuclei in anesthetized rats. J. Pharmacol. Exp. Ther. 214: 403-409, 1980.

67 Kvetnansky, R., Kopin, I.J., and Saavedra, J.M. Changes in epinephrine in individual hypothalamic nuclei after immobilization stress. Brain Res. 155: 387-390, 1978.

68 Laguzzi, R., Talman, W.T., and Reis, D.J. Serotonergic mechanisms in the nucleus tractus solitarius may regulate blood pressure and behaviour in the rat. Clin. Sci. 63: 323S-326S, 1982.

69 Lipski, J., McAllen, R.M., and Spyer, K.M. The Sinus nerve and baroreceptor input to the medulla of the cat. J. Physiol. (Lond.). 251: 61-78, 1975.

70 Little, R.A. and Oberg, B. Arterial baroreceptor function during elevation of intracranial pressure. Acta Physiol. Scand. 112: 27-32, 1981.

71 Loewy, A.D. and McKellar, S. Serotonergic projections from the ventral medulla to the intermediolateral cell column in the rat. Brain Res. 211: 146-152, 1981.

72 Lorenz, R.G., Saper, C.B., Wong, D.L., Ciaranello, R.D., and Loewy, A.D. Colocalization of substance P- and phenylethanolamine N-methyltransferase-like immunoreactivity in neurons of ventrolateral medulla that project to the spinal cord: Potential role in control of vasomotor tone. Neurosci. Lett. 55: 255-260, 1985.

73 Maley, B. and Elde, R. The ultrastructural localization of serotonin immunureactivity within the nucleus of the solitary tract of the cat. J. Neuroscience 2: 1499-1506, 1982.

74 Maruyama, S. Inhibition by topically applied clonidine and guanfacine on the pressor response to stimulation of the locus coeruleus in cats. Japan. J. Pharmacol. 31: 856-859, 1981.

75 Matsuura, S., Sakamoto, H., Hayashida, Y., and Kuno, M. Efferent discharges of sympathetic and parasympathetic nerve fibers during increased intracranial pressure in anesthetized cats in absence and presence of pressor response. Brain Res. 305: 291-301, 1984.

76 McNeill, T.H. and Sladek, J.R. Simultaneous monoamine histofluorescence and neuropeptide immunocytochemistry. I.Correlative distribution of catecholamine varicosities and magnocellular neurosecretory neurons in the rat supraoptic and paraventricular nuclei. J. Comp. Neurol. 193: 1023-1033, 1980.

77 Meeley, M.P., Ruggerio, D.A., Ishitsuka, T., and Reis, D.J. Intrinsic gamma-aminobutyric acid neurons in the nucleus of the solitary tract and the rostral ventrolateral medulla of the rat: an immunocytochemical and biochemical study. Neurosci. Lett. 58: 83-89, 1985.

78 Miyazaki, T., Coote, J.H., and Dun, N.J. Excitatory and inhibitory effects of epinephrine on neonatal rat sympathetic preganglionic neurons in vitro. Brain Res. 497: 108-116, 1989.

79 Morilak, D.A., Fornal, C.A., and Jacobs, B.L. Effects of physiological manipulations on locus coeruleus neuronal activity in freely moving cats. II. Cardiovascular challenge. Brain Res. 422: 24-31, 1987.

80 Murugaian, J., Sundaram, K., and Sapru, H. Cholinergic mechanisms in the ventrolateral medullary depressor area. Brain Res. 501: 355-363, 1989.

81 Neumayr, R.J., Hare, B.D., and Franz, D.N. Evidence for bulbospinal control of sympathetic preganglionic neurons by monoaminergic pathways. Life Sci. 14: 793-806, 1974.

82 Ogawa, M. Interactions between noradrenergic and srotonergic mechnanisms on the central regulation of blood pressure in the rat: a study using experimental central hypertension produced by chemical lesions of the locus coeruleus. Jpn. Circ. J. 42: 581-597, 1978.

83 Olpe, H.R., Berecek, K., Jones, R.S.G., Steinmann, M.W., Sonnenburg, C., and Hofbauer, K.G. Reduced activity of locus coeruleus neurons in hypertensive rats. Neurosci. Lett. 61: 25-29, 1985.

84 Pasztor, E., Fedina, L., Kocsis, B., and Berta, Z. Activity of peripheral sympathetic efferent nerves in experimental Subarachnoideal hemmorrhage. Part I: Observations at the time of intracranial hypertension. Acta. Neurochir. 79: 125-131, 1986.

85 Pfeiffer, A., Feurstein, G., Kopin, I.J., and Faden, A.I. Cardiovascular and respiratory effects of mu-, delta- and kappa-opiate agonists microinjected into the anterior hypothalamic brain area of awake rats. J. Pharmacol. Exp. Ther. 225: 735-741, 1983.

86 Pfeiffer, A., Feurstein, G., Zerbe, R.L., Faden, A.I., and Kopin, I.J. mu-Receptors mediate opioid cardiovascular effects at anterior hypothalamic sites through sympato-adrenomedullary and parasympathetic pathways. Endocrinology 113: 929-938, 1983.

87 Philippu, A. Involvement of cholinergic systems of the brain in the central regulation of cardiovascular functions. J. Auton. Pharmacol. 1: 321-330, 1981.

88 Philippu, A. Regulation of blood pressure by central neurotransmitters and neuropeptides. Rev. Physiol. Biochem. Pharmacol. 111: 1-115, 1988.

89 Philippu, A. Hypothalamic neurotransmitters: patterns of release and involvement in blood pressure regulation. In: Fleming, W.W., Langer, S.Z., Graefe, K.H. and Weiner, N. (eds), Neuronal and extraneuronal events in autonomic pharmacology. New York: Raven Press: 1984, 83-92.

90 Philippu, A., Dietl, H., and Sinha, J.N. Rise in blood pressure increases the release of endogenous catecholamines in the anterior hypothalamus of the cat. Naunyn-Schmiedeberg's Arch. Pharmacol. 310: 237-240, 1980.

91 Philippu, A., Przuntek, H., and Rosenberg, G. Superfusion of the hypothalamus with gamma-aminobutyric acid: effect on release of adrenaline and blood pressure. Naunyn-Schmiedeberg's Arch. Pharmacol. 276: 103-118, 1973.

92 Philippu, A. and Schartner, P. Inhibition by locally applied alpha-adrenoreceptor agents of the depressor response to stimulation of the anterior hypothalamus. Naunyn-Schmiedeberg's Arch. Pharmacol. 295: 1-7, 1976.

93 Pickel, V.M., Joh, T.H., and Reis, D.J. A serotonergic innervation of noradrenergic neurons in nucleus locus coeruleus: Demonstration by immunocytochemical localization of the transmitter specific enzymes tyrosine and tryptophan hydroxylase. Brain Res. 131: 197-214, 1977.

94 Pilowsky, P.M., Kapoor, V., Minson, J.B., West, M.J., and Chalmers, J.P. Spinal cord serotonin-and raised pressure after brainstem kainic acid injection. Brain Res. 366: 354-357, 1986.

95 Przuntek, H., Guimaraes, S., and Philippu, A. Importance of adrenergic neurons of the brain for the rise of blood pressure evoked by hypothalamic stimulation. Naunyn-Schmiedeberg's Arch. Pharmacol. 271: 311-319, 1971.

96 Przuntek, H. and Philippu, A. Reduced pressor responses to stimulation of the locus coeruleus after lesions of the posterior hypothalamus. Naunyn-Schmiedeberg's Arch. Pharmacol. 276: 119-122, 1973.

97 Pujol, J.F., Keane, P., McRae, A., Lewis, B.D., and Renaud, B. Biochemical evidence for serotonergic control of the locus coeruleus. In: Garratini, S., et al., eds. Interactions between putative neurotransmitters in the brain. New York: Raven Press: 1978, 401-410.

98 Punnen, S., Urbanski, R., Krieger, A.J., and Sapru, H.N. Ventrolateral medullary pressor area: Site of hypotensive action of clonidine. Brain Res. 422: 336-346, 1987.

99 Quintin, L., Gillon, J.Y., Ghignone, M., Renaud, B., and Pujol, J.F. Baroreflex-linked variations of catecholamine metabolism in the caudal ventrolateral medulla: An in vivo electrochemical study. Brain Res. 425: 319-336, 1987.

100 Reinhard, J.F. Jr. and Roth, R.H. Noradrenergic modulation of serotonin synthesis and metaboism. I. Inhibition by clonidine in vivo. J. Pharmacol. Exp. Ther. 114: 541-546, 1976.

101 Reis, D.J. The brain and hypertension: reflections on 35 years of inquiry into the neurobiology of circulation. Circulation 70 (Suppl. III): 31-45, 1984.

102 Renaud, B., Lambas-Senas, L., Fety, R., Colin, I., Astier, B., and Chamba, G. Biochemical pharmacology of brain adrenergic neurons: Comparison with noradrenergic neurons. In: Stolk, J.M., U'Prichard, D.C. and Fuxe, K., eds. Epinephrine in the central nervous system. New York: Oxford University Press: 1988, 140-153.

103 Ross, C.A., Ruggerio, D.A., Joh, T.H., Park, D.H., and Reis, D.J. Adrenaline synthesizing neurons in the rostral ventrolateral medulla: A possible role in tonic vasomotor control. Brain Res. 273: 356-361, 1983.

104 Ross, C.A., Ruggerio, D.A., Park, D.H., Joh, T.H., Sved, A.F., Pardal, J.F., Saavedra, J.M., and Reis, D.J. Tonic vasomotor control by the rostral ventrolateral medulla: Effect of electrical or chemical stimulation of the area containing C1 adrenaline neurons on arterial pressure, heart rate, and arterial plasma catecholamines and vasopressin. J. Neuroscience 4: 474-494, 1984.

105 Roth, K.A., Mefford, I.M., and Barchas, J.D. Epinephrine, norepinephrine, dopamine and serotonin: differential effects of acute and chronic stress on regional brain amines. Brain Res. 239: 417-424, 1982.

106 Routledge, C. and Marsden, C.A. Electrical stimulation of the C1 region of the rostral ventrolateral medulla of the rat increases mean arterial pressure and adrenaline release in the posterior hypothalamus. Neuroscience 20: 457-466, 1987.

107 Russell, G.V. The nucleus locus coeruleus dorsolateralis tegmenti. Tex. Rep. Biol. Med. 13: 939-988, 1955.

108 Saavedra, J.M., Grobecker, H., and Zivin, J. Catecholamines in the raphe nuclei in rat. Brain Res. 114: 339-345, 1976.

109 Saavedra, J.M., Kvetnansky, R., and Kopin, I.J. Adrenaline,noradrenaline and dopamine levels in specific brain stem areas of acutely immobilized rats. Brain Res. 160: 271-280, 1979.

110 Saavedra, J.M. and Torda, T. Increased brain stem and decreased hypothalamic adrenaline-forming enzyme after acute and repeated immobilization stress in the rat. Neuroendocrinology 31: 142-146, 1980.

111 Sadoshima, S., Thames, M., and Heistad, D. Cerebral blood flow during elevation of intracranial pressure: role of sympathetic nerves. Am. J. Physiol. 241 (Heart. Circ. Physiol. 10): H78-H84, 1981.

112 Sangdee, C. and Franz, D.N. Evidence for inhibition of sympathetic preganglionic neurons by bulbospinal epinephrine pathways. Neurosci. Lett. 37: 167-173, 1983.

113 Sauter, A.M., Baba, Y., Stone, E.A., and Goldstein, M. Effect of stress and of phenylethanolamine-N-methyltransferase inhibition on central norepinephrine and epinephrine levels. Brain Res. 144: 415-419, 1978.

114 Saxena, A.K., Saksena, A.K., Agnihotri, M.S., Vrat, S., Tangri, K.K., and Bhargava, K.P. Cardiovascular responses elicited by microinjection of monoamines into mesencephalic nucleus dorsalis raphe in cats. Naunyn-Schmiedeberg's Arch. Pharmacol. 329: 141-145, 1985.

115 Seller, H. and Ilert, M. The localization of the first synapse in the carotid sinus baroreceptor reflex pathway and its alteration of the afferent input. Pflugers Arch. 306: 1-19, 1969.

116 Sinha, J.N., Dietl, H., and Philippu, A. Effect of a fall of blood pressure on the release of catecholamines in the hypothalamus. Life Sci. 26: 1751-1760, 1980.

117 Smits, J.F. and Struyker-Boudier, H.A. Intrahypothalamic serotonin and cardiovascular control in rats. Brain Res. 111: 422-427, 1976.

118 Smits, J.F., Van Essen, H., and Struyker-Boudier, A.J. Serotonin-mediated cardiovascular responses to electrical stimulation of the raphe nuclei in the rat. Life Sci. 23: 173-178, 1978.

119 Snider, R.S. and Niemer, W.T. A stereotaxic atlas of the cat brain. Chicago, Ill.: Univ. of Chicago Press. 1961.

120 Stoner, H.B. and Elson, P.M. The effect of injury on monoamine concentrations in the rat hypothalamus. J. Neurochem. 81: 1837-1846, 1971.

121 Stoner, H.B. and Hunt, A. The effect of trauma on the activity of central noradrenergic neurons. Brain. Res. 112: 337-346, 1976.

122 Struyker-Boudier, H., Smeets, G., Brouwer, G., and Van Rossum, J. Hypothalamic alpha-adrenergic receptors in cardiovascular regulation. Neuropharmacology 13: 837-841, 1974.

123 Sved, A.F. Stimulation of the noradrenergic cells of the locus coeruleus in rat decreases blood pressure. Soc. Neurosci. Abstr. 11: 193, 1985.

124 Svensson, T.H. and Thoren, P. Brain noradrenergic neurons in the locus coeruleus: inhibition by blood volume load through vagal afferents. Brain Res. 172: 174-178, 1979.

125 Tadepalli, A.S., Mills, E., and Schanberg, S.M. Central depression of carotid baroreceptor response, arterial pressure and heart rate by 5-hydroxytryptophan: Influence of supracollicular areas of the brain. J. Pharmacol. Exp. Ther. 202: 310-319, 1977.

126 Talman, W.T., Perrone, M.H., and Reis, D.J. Evidence for L-glutamate as a neurotransmitter of baroreceptor afferent nerve fibers. Science 209: 813-815, 1980.

127 Van Wylen, D.G.L. and D'Alecy, L.G. Regional blood flow distribution during the Cushing response: alterations with adrenergic blockade. Am. J. Physiol. 248 (Heart Circ. Physiol. 17): H98-H108, 1985.

128 Vlahakos, D. and Gavras, I. Gavras H. Alpha-adrenoreceptor agonists applied in the area of the nucleus tractus solitarii in the rat: effect of anesthesia on cardiovascular responses. Brain Res. 347: 372-375, 1985.

129 Wang, Q. and Li, P. Inhibition of baroreflex following microinjection of GABA or morphine into the nucleus tractus solitarii in rabbits. J. Autonom. Nerv. Syst. 25: 165-172, 1988.

130 Ward, D.G. Activation of solitary nucleus neurons from the locus coeruleus and vicinity. Soc. Neurosci. Abstr. 1: 424, 1975.

131 Ward, D.G. and Gunn, C.G. Locus coeruleus complex: elicitation of a pressor response and a brain stem region necessary for its occurence. Brain Res. 107: 401-406, 1976.

132 Willette, R.N., Barcas, P.P., Krieger, A.J., and Sapru, H.N. Endogenous GABAergic mechanisms in the medulla and the regulation of blood pressure. J. Pharmacol. Exp. Ther. 230: 34-39, 1984.

133 Willette, R.N., Krieger, A.J., Barcas, P.P., and Sapru, H.N. Medullary gamma-aminobutyric acid (GABA) receptors and the regulation of blood pressre in the rat. J. Pharmacol. Exp. Ther. 226: 893-899, 1983.

134 Wolf, W.A., Kuhn, D.M., and Lovenberg, W. Blood pressure responses to local application of serotonergic agents in the nucleus tractus solatarii. Eur. J. Pharmacol. 69: 291-299, 1981.

135 Yamada, K.A., Norman, W.P., Hamosh, P., and Gillis, R.A. Medullary ventral surface GABA receptors affect respiratory and cardiovascular function. Brain Res. 248: 71-78, 1982.

136 Yukimura, T., Fuxe, K., Ganten, D., Andersson, K., Harfstrand, A., Unger, T., and Agnati, L.F. Acute sino-aortic denervation in rats produces a selective increase of adrenaline turnover in the dorsal midline area of the caudal medulla oblogata and a reduction of adrenaline levels in the anterior and posterior hypothalamus. Eur. J. Pharmacol. 69: 361-365, 1981.

Section E: Effects of CM_1 Ganglioside Treatment On Behavioral Recovery After A Missile Wound To The Brain

PAGE PURPOSELY BLANK

INTRODUCTION

Gangliosides are sialic acid-containing glycosphingolipids found in high concentrations in the CNS, especially in synaptic membranes. GM1 ganglioside treatment has been demonstrated to significantly enhance behavioral and neurochemical recovery from discrete neurotoxic and mechanical lesions (1-4, 6-12). Moreover, the GM1 gangliosides can be given exogenously and do not possess any known toxicological effects (5). Therefore, the encouraging data from the aforementioned discrete lesions experiments, the ease in which treatment can be started and continued, and the lack of toxicological effects all make GM1 gangliosides an ideal drug to initially test in our brain wounding model. The present study was undertaken in order to determine if GM_1 gangliosides treatment would affect behavioral recovery from specifically from a missile wound of the brain.

METHODOLOGY

A population of cats (both sexes) were matched according to weight into pairs. The matching by pairs was deemed necessary in order to preclude weight as a factor in the behavioral motor tests, especially beam balance performance. Each pair of cats were injured (0.9 J) then randomly assigned to either the control group or drug treatment group. Control cats received saline I.P) and drug-treated cats received GM_1 ganglioside (20 mg/kg, I.P.). The IP injections began approximately 10 mins. after injury, then daily for the next 10 days. When an injection day coincided with a test day, the injection were given one hour or more prior to testing. The cats were tested by a "blind" rater, i.e. the rater did not know to which of two groups the cats were assigned to. Testing began third day post-injury, then every third day thereafter for 30 days; then weekly for 5 weeks (65 days post-injury).

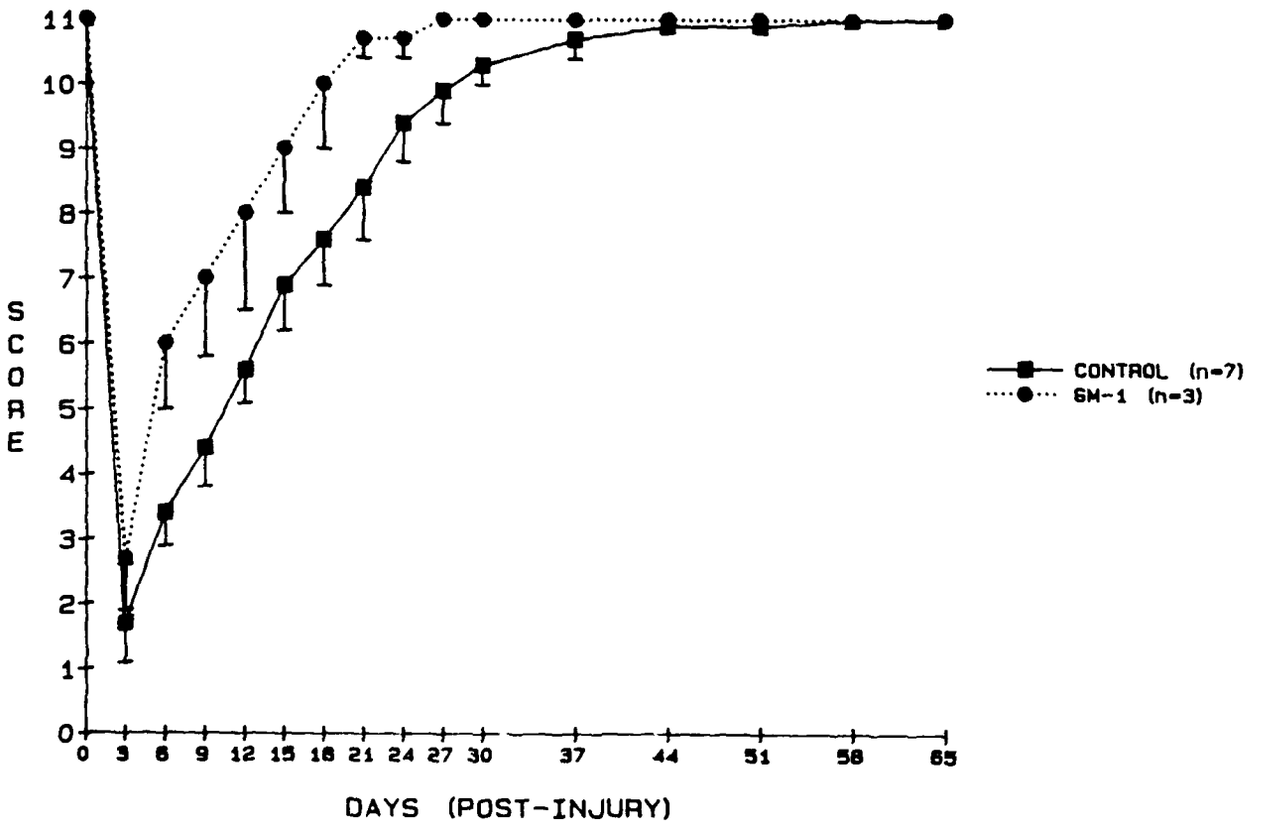
The cats were scored according to the criteria previously described in detail in our Annual report dated april 27, 1989. The protocol and procedure for handling and treating cats intended to survive, as well as a scoring sheet and criteria, are included at the end of this report.

RESULTS

Because of inadvertent pregnancies, three cats had to be eliminated from the GM_1 ganglioside treatment group along with one atypically affected cat. This resulted in an n=7 for the control group and only an n=3 for the GM_1 ganglioside treated group. Therefore, the results are not conclusive because the total number of subjects (n) for each group is too small to apply the appropriate statistical evaluation at this time.

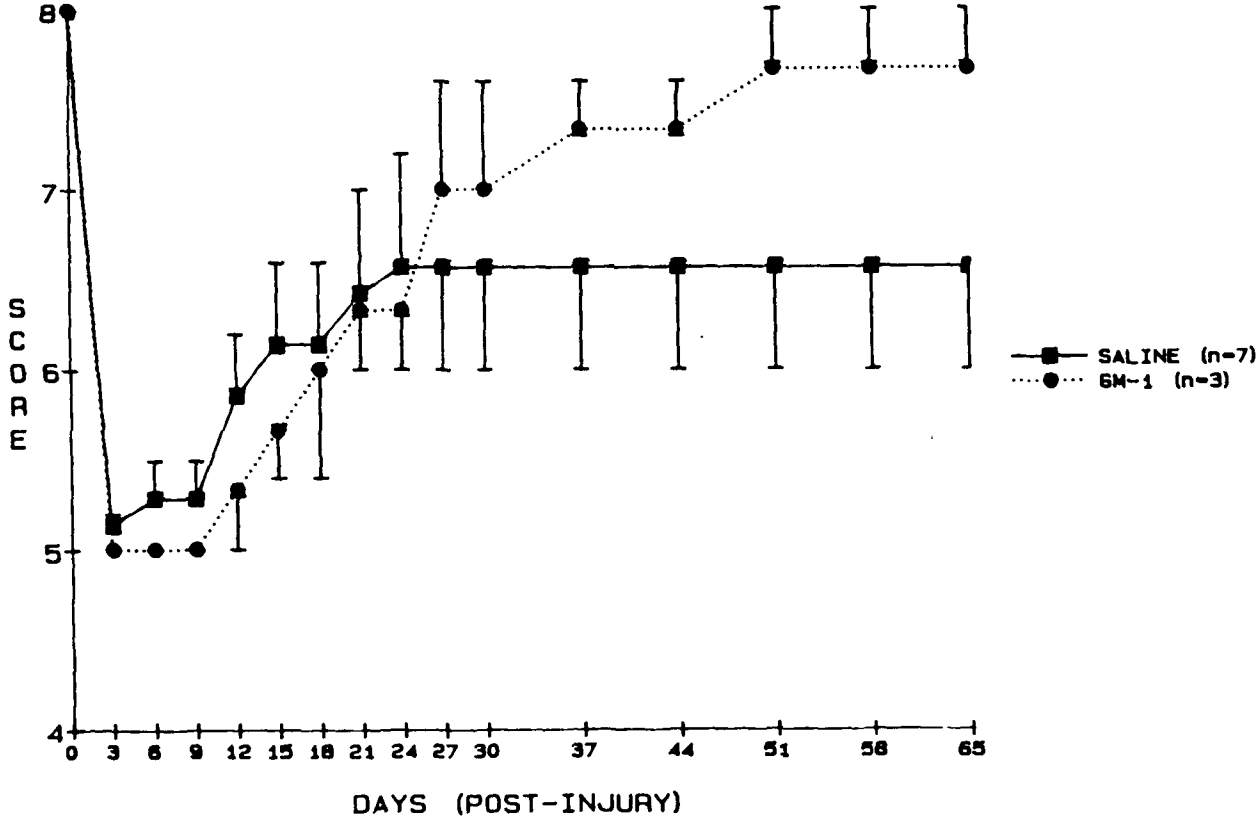
The data, however, are encouraging. Beam balance performance appeared to be enhanced in the GM_1 ganglioside-treated cats (Fig. 1; Table 1).

Fig. 1. BEAM BALANCE PERFORMANCE OF INJURED CATS



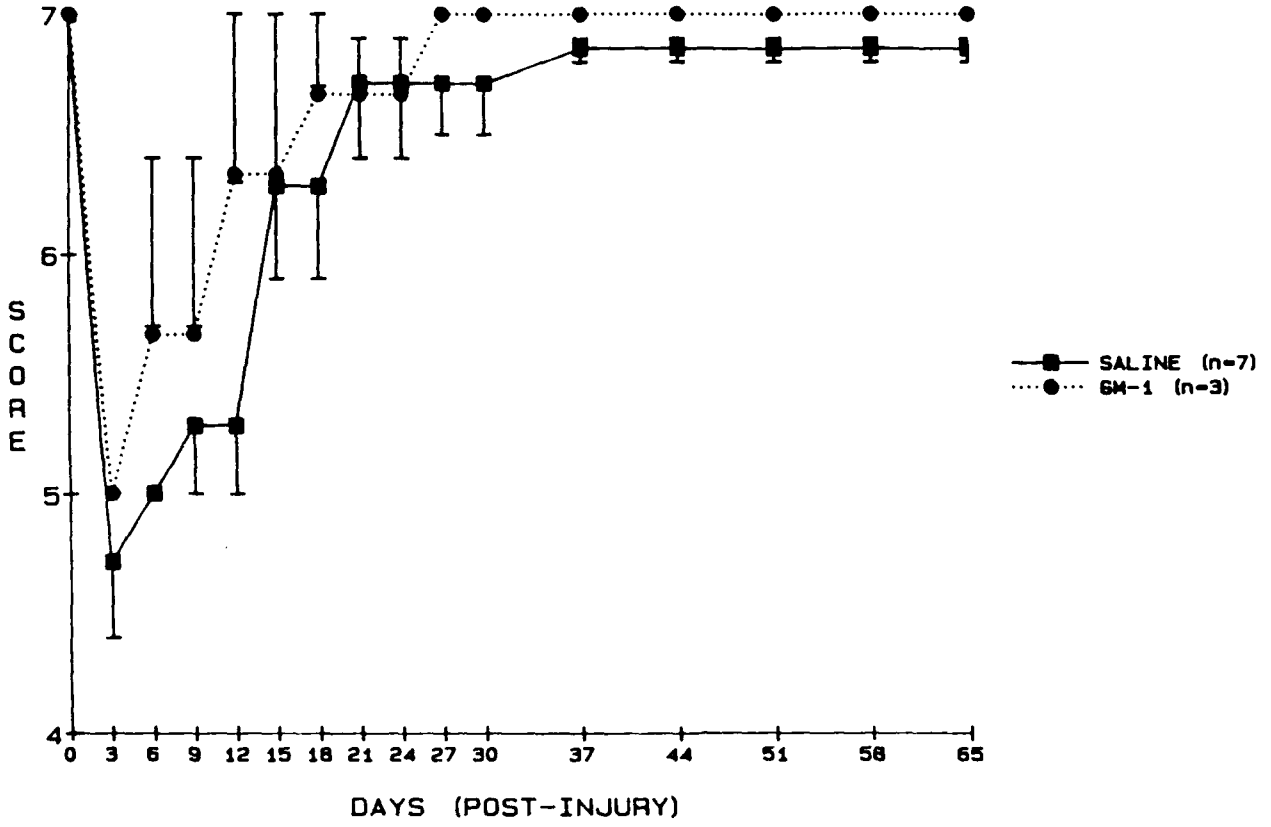
Non-visual placing performance of the GM_1-treated cats initially did not appear to be different than non-treated cats. But the GM_1-treated cats ultimately appeared to have a better recovery in this modality (days 37-65) (Fig. 2; table 2).

Fig. 2. NON-VISUAL PLACING PERFORMANCE OF INJURED CATS



Visual placing performance appeared to be initially more improved due to GM_1 ganglioside treatment (days 3-12) (Fig. 3; Table 3).

Fig. 3. VISUAL PLACING PERFORMANCE OF INJURED CATS



DISCUSSION

Because of the insufficient number of animals included in the study, especially in the GM_1 ganglioside-treated group, these results must be considered preliminary. Nevertheless, the results are very encouraging and suggest that GM_1 gangliosides may speed up and even enhance recovery of neural functioning following a missile wound to the brain.

The accelerated recovery in beam balance performance of GM_1-treated cats may be related to their initially better visual placing performance or to their ultimately better non-visual placing performance, or both. Further testing is definitely warranted

We have reported our results to Fidia Pharmaceuticals (Abano Terme, Italy) who graciously supplied the GM_1 ganglioside used in this study. We have also requested and received additional GM_1 ganglioside for further testing in our penetrating brain injury model and anticipate completing the study in the Fall of 1989 or Winter 1990 if Congress allows us to proceed with our program.

Table 1. BEAM BALANCE PERFORMANCE OF INJURED CATS

	PRE	3d	6d	9d	12d	15d	18d	21d
SALINE								
RB2	11.0	2.0	4.0	5.0	7.0	7.0	7.0	7.0
C210	11.0	0.0	2.0	4.0	5.0	7.0	7.0	9.0
C1686	11.0	2.0	2.0	2.0	4.0	7.0	8.0	10.0
C207	11.0	1.0	4.0	4.0	5.0	5.0	6.0	7.0
C1627	11.0	0.0	2.0	4.0	4.0	4.0	5.0	5.0
C15	11.0	5.0	5.0	5.0	7.0	10.0	10.0	10.0
C18	11.0	2.0	5.0	7.0	7.0	8.0	10.0	11.0
MEAN	11.0	1.7	3.4	4.4	5.6	6.9	7.6	8.4
+/-SEM	0.0	0.6	0.5	0.6	0.5	0.7	0.7	0.8
GM-1								
C14	11.0	2.0	7.0	9.0	10	10.0	11.0	11.0
C2	11.0	4.0	7.0	7.0	9.0	10.0	11.0	11.0
C10	11.0	2.0	4.0	5.0	5.0	7.0	8.0	10.0
MEAN	11.0	2.7	6.0	7.0	8.0	9.0	10.0	10.7
+/-SEM	0.0	0.7	1.0	1.2	1.5	1.0	1.0	0.3

	24d	27d	30d	37d	44d	51d	58d	65d
SALINE								
RB2	10.0	10.0	10.0	11.0	11.0	11.0	11.0	11.0
C210	10.0	10.0	10.0	11.0	11.0	11.0	11.0	11.0
C1686	10.0	10.0	11.0	11.0	11.0	11.0	11.0	11.0
C207	9.0	10.0	10.0	11.0	11.0	11.0	11.0	11.0
C1627	6.0	7.0	9.0	9.0	10.0	10.0	11.0	11.0
C15	10.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0
C18	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0
MEAN	9.4	9.9	10.3	10.7	10.9	10.9	11.0	11.0
+/-SEM	0.6	0.5	0.3	0.3	0.1	0.1	0.0	0.0
GM-1								
C14	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0
C2	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0
C10	10.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0
MEAN	10.7	11.0	11.0	11.0	11.0	11.0	11.0	11.0
+/-SEM	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 2. NON-VISUAL PLACING PERFORMANCE OF INJURED CATS

	PRE	3d	6d	9d	12d	15d	18d	21d
SALINE								
RB2	8.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
C210	8.0	5.0	5.0	5.0	7.0	7.0	7.0	8.0
C1686	8.0	5.0	6.0	6.0	6.0	7.0	7.0	8.0
C207	8.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
C1627	8.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
C15	8.0	6.0	6.0	6.0	7.0	8.0	8.0	8.0
C18	8.0	5.0	5.0	5.0	6.0	6.0	6.0	6.0
MEAN	8.0	5.1	5.3	5.3	5.9	6.1	6.1	6.4
+/-SEM	0.0	0.1	0.2	0.2	0.3	0.5	0.5	0.6
GM-1								
G14	8.0	5.0	5.0	5.0	5.0	5.0	5.0	6.0
G2	8.0	5.0	5.0	5.0	6.0	6.0	7.0	7.0
G10	8.0	5.0	5.0	5.0	5.0	6.0	6.0	6.0
MEAN	8.0	5.0	5.0	5.0	5.3	5.7	6.0	6.3
+/-SEM	0.0	0.0	0.0	0.0	0.3	0.3	0.6	0.3

	24d	27d	30d	37d	44d	51d	58d	65d
SALINE								
RB2	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
C210	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
C1686	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
C207	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
C1627	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
C15	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
C18	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0
MEAN	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6
+/-SEM	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
GM-1								
G14	6.0	6.0	6.0	7.0	7.0	8.0	8.0	8.0
G2	7.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
G10	6.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0
MEAN	6.3	7.0	7.0	7.3	7.3	7.7	7.7	7.7
+/-SEM	0.3	0.6	0.6	0.3	0.3	0.3	0.3	0.3

Table 3. VISUAL PLACING PERFORMANCE OF INJURED CATS

	PRE	3d	6d	9d	12d	15d	18d	21d
SALINE								
RB2	7.0	5.0	5.0	5.0	5.0	6.0	6.0	6.0
C210	7.0	5.0	5.0	5.0	5.0	7.0	7.0	7.0
C1686	7.0	5.0	5.0	5.0	5.0	7.0	7.0	7.0
C207	7.0	5.0	5.0	5.0	5.0	5.0	5.0	6.0
C1627	7.0	3.0	5.0	5.0	5.0	5.0	5.0	7.0
C15	7.0	5.0	5.0	7.0	7.0	7.0	7.0	7.0
C18	7.0	5.0	5.0	5.0	5.0	7.0	7.0	7.0
MEAN	7.0	4.7	5.0	5.3	5.3	6.3	6.3	6.7
+/-SEM	0.0	0.3	0.0	0.3	0.3	0.4	0.4	0.2
GM-1								
G14	7.0	5.0	7.0	7.0	7.0	7.0	7.0	7.0
G2	7.0	5.0	5.0	5.0	7.0	7.0	7.0	7.0
G10	7.0	5.0	5.0	5.0	5.0	5.0	6.0	6.0
MEAN	7.0	5.0	5.7	5.7	6.3	6.3	6.7	6.7
+/-SEM	0.0	0.0	0.7	0.7	0.7	0.7	0.3	0.3

	24d	27d	30d	37d	44d	51d	58d	65d
SALINE								
RB2	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
C210	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0
C1686	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0
C207	6.0	6.0	6.0	7.0	7.0	7.0	7.0	7.0
C1627	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0
C15	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0
C18	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0
MEAN	6.7	6.7	6.7	6.9	6.9	6.9	6.9	6.9
+/-SEM	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.1
GM-1								
G14	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0
G2	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0
G10	6.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0
MEAN	6.7	7.0	7.0	7.0	7.0	7.0	7.0	7.0
+/-SEM	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0

REFERENCES

1 Agnati, L.F., Fuxe, K., Benfenati, F., Zoli, M., Owman, C., Diemers, N.H., Kahrstrom, J.K., Toffano, G., and Cimino, M. Effect of ganglioside GM1 on striatal glucose metabolism, blood flow, and protein phosphorylation of the rat. Acta Physiol. Scand. 125: 43-53, 1985.

2 Agnati, L.F., Fuxe, K., Zoli, M., Davalli, P., Corti, A., Zini, I., and Toffano, G. Effects of neurotoxic and mechanical lesions of the mesostriatal dopamine pathway on striatal polyamine levels in the rat: Modulation by chronic GM1 ganglioside treatment. Neurosci. Lett. 61: 339-344, 1985.

3 Fass, B. and Ramirez, J.J. Effect of ganglioside treatments on lesion-induced behavioral impairments and sprouting in the CNS. J. Neurosci. Res. 12: 445-458, 1984.

4 Hadjiconstantinou, M., Rosseti, Z.L., Paxton, R.C., and Neff, N.H. Administration of GM1 ganglioside restores the dopamine content in striatum after chronic treatment with MPTP. Neuropharmacol. 25(9): 1075-1077, 1986.

5 Heywood, R., Chesterman, H., Hunter, B., Palmer, A.K., Majeed, S.K., and Prentice, D.E. The toxicology of a ganglioside extract (cronassial). Toxicol. Lett. 15: 275-282, 1983.

6 Karpiak, S.E. Ganglioside treatment improves recovery of alternation behavior after unilateral entorhinal cortex lesion. Exp. Neurol. 81: 330-339, 1983.

7 Mahadik, S.P., Vilim, F., Korenovsky, A., and Karpiak, S.E. GM1 ganglioside protects nucleus basalis from excitotoxin damage: Reduced cortical cholinergic losses and animal mortality. J. Neurosci. Res. 20: 479-483, 1988.

8 Sabel, B.A., Dunbar, G.L., and Stein, D.G. Gangliosides minimize behavioral deficits and enhance structural repair after brain injury. J. Neurosci. Res. 12: 429-443, 1984.

9 Sabel, B.A., Slavin, M.D., and Stein, D.G. GM1 ganglioside treatment facilitates behavioral recovery from bilateral brain damage. Science 225: 340-342, 1984.

10 Toffano, G., Agnati, L.F., Fuxe, K., Aldinio, C., Consolazione, A., Valenti, G., and Savoini, G. Effect of GM1 ganglioside treatment on the recovery of dopaminergic nigro-striatal neurons after different types of lesion. Acta Physiol. Scand. 122: 313-321, 1984.

11 Toffano, G., Savoini, G., Aporti, F., Calzolari, S., Consolazione, A., Maura, G., Marchi, M., Raiteri, M., and Agnati, L.F. The functional recovery of damaged brain: The effect of GM1 monoganglioside. J. Neurosci. Res. 12: 397-408, 1984.

12 Toffano, G., Savoini, G., Moroni, F., Lombardi, G., Calza, L., Agnati, and L.F. GM1 ganglioside stimulates the regeneration of dopaminergic neurons in the central nervous system. Brain Res. 261: 163-166, 1983.

STANDARD PROTOCOL FOR CATS INTENDED TO SURVIVE AND USED FOR BEHAVIORAL AND DRUG TESTING

1. Cat is weighed and appropriate dose (40 mg/kg,IP) of pentobarbital is administered.

2. The weight of the cat is entered into a computerized record.

3. ALL SURGICAL PROCEDURES ARE PERFORMED UNDER STERILE CONDITIONS.

4. Adequacy of anaesthesia is evaluated by:
   a. Cessation of limb withdrawal from pinch (using thumb and index finger) between toes.
   b. Abolition of corneal reflex (tip of paper tissue touched to cornea).

   Once depth of anaesthesia is deemed adequate, one arterial cannula is implanted after treatment of the incision area with local anaesthetic (2% xylocaine). IF THE CAT SHOWS ANY SIGN OF DISCOMFORT DURING THE CANNULA IMPLANTATION PROCEDURE, GENERAL ANAESTHESIA IS SUPPLEMENTED WITH PENTOBARBITAL (6.5 mg) VIA THE ARTERIAL CANNULA.

   RATIONALE FOR THE IMPLANTATION OF AN ARTERIAL CANNULA ONLY:

   An arterial cannula is inserted in the right rear leg to measure the mean arterial blood pressure (MABP). Since the the cannulated artery is eventually tied off, no venous cannula is inserted into the right rear leg in order not to compromise the venous return from the same leg. The left rear leg is not cannulated at all because it becomes paretic following injury Supplemental anaesthethic can be safely given through an arterial cannula.

5. An endotracheal tube, smeared with topical anaesthetic (2% xylocaine jelly) is inserted after application of local anaesthetic (0.5 ml 2% xylocaine) to the epiglottis.

6. Cat is mounted in the stereotaxic frame, then:
   a. MABP transducer is attached to the arterial cannula.
   b. Endotracheal tube is attached to an end tidal CO_2 monitor.
   c. The depth of anaesthesia is rechecked using the 2 criteria described above as well as the MABP and respiratory rates.
   d. The cat may receive supplemental pentobarbital based on the above four criteria as a group. This is a judgement call, as no one criterion is a perfect indicator of depth of anaesthesia. Supplements are given in aliquots of 6.5mg, through the arterial cannula.

7. Surgery is performed:
   a. An area of the head is shaved, and a 5 cm. scalp incision is made.
   b. The anterior wall (1 cm.X 1 cm.) of the right frontal sinus is removed.
   c. If the cat shows any signs of discomfort during any of these procedures, supplemental pentobarbital is given as required (6.5 mg, via the arterial cannula).

   RATIONALE FOR NOT INSERTING AN ICP PROBE:
   The ICP probe is not inserted into these cats because:

   a) Prior results indicate that the injury caused by a 0.9 Joule missile wound causes only a very modest increase in intracranial pressure, on the average, (20 mm Hg vs 6 mm Hg-control). This modest rise is not at all life threatening.

   b) The insertion of the ICP probe could possibly lead to other problems, such as, additional brain injury caused by tearing by the ICP probe due to movement of the brain against the stationary probe after the missile injury. Thus, insertion of the ICP probe would add nothing to the experiment except the possibility of added nonspecific damage to the brain.

8. ALL cats used in behavioral studies are injured by a 0.9 Joule missile ONLY, because there is a greater chance of survival (approx. 70%) at this missile energy level

9. After injury, the scalp incision is sutured. The femoral arterial cannula removed, the artery tied off and the groin incision sutured.
   Once assured of adequate respirations, the cat is suctioned through the endotracheal tube and the endotracheal tube removed.

10. The cat is given antibiotics (penecillin G, 300,000 U,IM) and topical anaesthetic (2% lidocaine jelly) applied to the sutures (scalp and groin).

11. The cat is returned to the animal care facility and covered with a blanket. AS PART OF OUR STANDARD PROTOCOL, THE VETERNARIAN IS NOTIFIED AND LACTATED RINGERS SOLUTION (180cc) IS ADMINISTERED THE NEXT MORNING. ADDITIONAL ANTIBIOTICS ARE ALSO ADMINISTERED BY THE VETERNARIAN FOR THE FIRST 3 DAYS POST-INJURY.

12. Topical anaesthetic (2% lidocaine jelly) is applied to the

sutures (scalp and groin) once daily for the first 3 days post-injury.

13. The cat is observed daily to determine if the cat is eating and drinking ad lib. If the cat is not able to eat and drink, the VETERNARIAN IS NOTIFIED and lactated ringers (180cc) is given.
Most cats are eating and drinking ad lib the SECOND day post-injury. ALL cats are eating and drinking by day three post-injury.

14. ALL behavioral tests begin on day THREE post-injury and retesting is performed every third day for 30 days, then weekly thereafter for 4 more weeks.

15. NONE of the behavioral tests are traumatic to the cats. The cats are INDUCED to walk the balance beam using canned tuna fish as a reinforcer. Tuna is an excellent reinforcer because NO food deprivation is needed for its reinforcer qualities.

16. NOTE: NO PARALYZING DRUGS ARE GIVEN AT ANY TIME DURING THESE EXPERIMENTS.

DATES 198

CAT #

SENSORY															
Score (1-5)															
LEVEL OF CONSCIOUSNESS															
Score (1-5)															
VISION															
Field Cut:															
Score (R2,R1)															
(L2,L1)															
Pupillary:															
Score (R2,R1)															
(L2,L1)															
AUDITORY															
Orientation (R2,L2)															
NO Orient. (R1,L1)															
MOTOR FUNCTION															
Score (1-6)															
NB right,left fore/hind limb															
CIRCLING															
Score (Y=1,N=3)															
BEAM BALANCE															
Score (0-11)															
GRID BEAM															
Score (# missteps)															
VISUAL PLACING															
(a) Both (1-3)															
(b) R (1-2)															
(c) L (1-2)															
NON-VISUAL PLACING															
(a) Right (1-4)															
(b) Left (1-4)															
SENSORY MOTOR															
Latencies:															
Right fore															
10															
40															
60															
Left fore															
10															
40															
60															

COMMENTS:

BEHAVIORAL RESPONSES TO PENETRATING BRAIN INJURIES

SENSORY FUNCTION

Responds appropriately to tactile and noxious stimuli	5
Responds appropriately to noxious stimuli only	4
Inappropriate response to noxious limb stimulation	3
Reflex response to noxious stimulation	2
No response to noxious stimulation	1

LEVEL OF CONSCIOUSNESS

Awake and alert	5
Awake and alert with a lack of spontaneous movement	4
Drowsy, responds only to noxious stimulation	3
Stuporous, minimal response to noxious stimuli	2
Comatose	1

VISION

Field cut (pencil movement-right or left): if normal	2
deficit (r or l)	1
Pupillary response: Unilaterally reactive to light	2
Unilaterally unresponsive to light	1

AUDITORY

Orientation of head to appropriate side of auditory stimulus on the right or left side	2
No orientation	1

NB Example: L1--no orientation to stimulus on left side.

MOTOR FUNCTION

Walks with normal gait - no apparent neurological deficit	6
Walks with abnormal gait, has mild hemiparesis	5
Barely walks with moderate hemiparesis	4
Unable to walk with moderate hemiparesis	3
Unable to walk with severe hemiparesis	2
Unable to walk with hemiplegia	1

CIRCLING

Shows no overt signs of circling	3
Displays overt circling to the right (note only if to left)	1

VISUAL PLACING

(a) Both forelimbs free:		
	Places BOTH limbs	3
	Places ONE limb (note L or R)	2
	NO placing	1
(b) Right limb restrained:		
	Able to place left	2
	Unable to place left	1
(c) Left limb restrained:		
	Able to place right	2
	Unable to place right	1

NONVISUAL PLACING

Placing to guard hair contact	4
Placing requires pressure on paw	3
Placing requires forced paw deflection <45 degrees	2
No placing i.e. > 45 degree paw deflection	1

SENSORY MOTOR

Latency to withdraw each individual paw from water at 10, 40 and 60 degrees C. Maximum time of 60 sec. for 10 and 40, while the maximum time at 60 is 5 sec.

NOTE: Whenever the cat scores perfectly for three successive testing periods ,that particular test can be discontinued, as the cat is considered normal or recovered.

BEAM BALANCE SCORING

11 Normal traverse walking and turning.

10 Normal traverse walking and can turn one way EASILY and the other way with difficulty.

9 Normal traverse walking and can turn one way EASILY, but turning the other way is impossible.

8 Normal traverse walking and can turn both ways, but with difficulty.

7 Normal traverse walking and can turn one way with difficulty, but the other way is impossible.

6 Normal traverse walking, but turning is impossible.

5 Difficulty in traversing and turning is impossible.

4 Can take only 1 step w/o falling.

3 Can place BOTH affected paws on the horizontal surface of the beam, but cannot take one step w/o falling or does not attempt to walk.

2 Can place ONE affected paw on the horizontal surface of the beam, but cannot take one step w/o falling or does not attempt to walk.

1 Can maintain balance, but cannot place BOTH affected paws on the horizontal surface of the beam and does not attempt to walk.

0 Cannot stay on the beam w/o assistance.

NOTE: Scores 0-4 are more applicable if the cat requires placement onto the beam in order to test.
Scores 4-11 are more applicable if the cat will walk the beam of its own accord or for tuna.